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## Revealing how intra- and inter-annual variability of carbon uptake (GPP) affects wood cell biomass in an eastern white pine forest

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E-mail: [daniele.castagneri@unipd.it](mailto:daniele.castagneri@unipd.it)**Keywords:** carbon uptake, cell wall area, eddy covariance, gross primary productivity, tree rings, woody biomass, xylem anatomySupplementary material for this article is available [online](#)

## Abstract

Forests are major terrestrial carbon (C) sinks and play a crucial role in climate change mitigation. Despite extensive studies on forest C sequestration, the relationship between seasonal C uptake and its allocation to woody biomass is poorly understood. Here we used a novel dendro-anatomical approach to investigate the relationships between climate variability, C uptake, and woody biomass growth in an 80 year-old eastern white pine (*Pinus strobus*) plantation forest in Ontario, Canada. We used eddy covariance (EC) gross primary productivity (GPP) from 2003–2018 and woody biomass estimated from chronologies of cell wall area (CWA, a proxy for C storage in individual wood cells) and ring wall area (RWA) for earlywood (EW) and latewood (LW) from 1970–2018. Warm temperatures in early spring and high precipitation in mid-spring and summer positively and strongly affected GPP, while high temperature and high vapor pressure deficit in the summer had a negative effect. From 2003 to 2018, there was a steady increase in both GPP and woody cell biomass. Moreover, we found strong positive correlations between GPP and CWA both in EW (May–July GPP,  $r = 0.65$ ) and LW (July–August GPP,  $r = 0.89$ ). Strong positive correlations were also found between GPP and RWA both in EW and LW (April–September,  $r \geq 0.79$ ). All these associations were stronger than the association between annual GPP and tree-ring width ( $r = 0.61$ ) used in previous studies. By increasing the resolution of tree-ring analysis to xylem-cell level, we captured intra-annual variability in biomass accumulation. We demonstrated a strong control of seasonal C assimilation (source) over C accumulation in woody biomass at this site. Coupling high-resolution EC fluxes (GPP) and wood anatomical measurements can help to reduce existing uncertainties on C source-sink relationships, opening new perspectives in the study of the C cycle in forests.

## 1. Introduction

Better understanding of the terrestrial carbon (C) cycle is one of the most pressing scientific needs of our time (Cabon *et al* 2022). Despite the understanding of

basic mechanisms of photosynthesis since the mid-20th century (Rabinowitch 1951), most aspects of the biospheric C cycle are not completely understood (Dusenge *et al* 2019). Forests absorb a significant portion of CO<sub>2</sub> emitted by human activities, thus

mitigating climate warming (Bonan 2008, Pan *et al* 2011). However, how climate influences tree primary productivity, and how this will affect the capacity of forests to act as a C sink, remains uncertain (Baldocchi *et al* 2018, Keenan and Williams 2018).

Among others, the relationships between photosynthesis (C source) and C allocation to wood structure (above-ground woody biomass growth) are fundamental in understanding the terrestrial C cycle (Fatichi *et al* 2014). Empirical studies have demonstrated that these two activities have different sensitivity to climate variability (Körner 2003), and there is a large uncertainty about how much acquired C is actually allocated to above-ground woody biomass (Pappas *et al* 2020, Cabon *et al* 2022). Most land surface schemes of the global climate models use simple C allocation approaches to estimate tree biomass growth, which may introduce large uncertainty in their simulations. Therefore, a better understanding and representation of C allocation to above-ground woody biomass is a fundamental step forward in improving the predictive capabilities of these models, which in turn strongly influence global environmental policies (Montané *et al* 2017, Jung *et al* 2020, Babst *et al* 2021).

Over the last two decades, several studies have used multiple approaches to investigate the link between ecosystem primary productivity and secondary forest growth (Wang *et al* 2004, Vicente-Serrano *et al* 2020). More recently, the increasing availability of multi-year records of ecosystem productivity measurements from eddy-covariance (EC) stations in forests has allowed researchers to assess the association between inter-annual variability of above-ground woody biomass accumulation and gross or net primary productivity (GPP and NPP). Such associations have been very variable among different sites (Babst *et al* 2014) and studies, ranging from not significant (Rocha *et al* 2006, Delpierre *et al* 2016, Pappas *et al* 2020, Oddi *et al* 2022) to highly positively significant (Xu *et al* 2017, McKenzie *et al* 2021, Metsaranta *et al* 2021). In addition, Teets *et al* (2018) and Tei *et al* (2019) found significant positive associations, but observed that annual tree biomass increment lagged one year behind net ecosystem production (NEP) and GPP, respectively. Such a lag may be related to variable C allocation patterns during stress events in the growing season. Therefore, studies that consider different time scales and more refined estimates of above-ground woody biomass accumulation in trees are necessary to better understand the causes behind the decoupling between C absorption and tree biomass growth, and how these relate to climate variability and extreme events (Lee *et al* 2021, Arain *et al* 2022).

Several studies conducted in forest EC sites have used tree-ring width (TRW) to estimate biomass

growth (McKenzie *et al* 2021, Oddi *et al* 2022). Tree rings are a unique source of information, providing annually resolved measures of tree radial growth over long time periods (Speer 2010). However, the temporal scale of TRW, an integrated metric of annual tree growth (Kannenber *et al* 2020), scarcely matches the high resolution of EC estimates of productivity. Furthermore, a limitation of using TRW to estimate biomass variability is the (major) assumption of constant wood density, i.e. lack of considering inter-annual variability of wood anatomy (Björklund *et al* 2017), which can lead to relevant biases (Cuny *et al* 2015, Pretzsch *et al* 2018, Vannoppen *et al* 2018). Other methods to assess tree growth, such as stem circumference increment (Delpierre *et al* 2016) and xylogenesis (Krejza *et al* 2022) monitoring, are more temporally detailed and provide information on intra-annual wood formation processes and phenology, but lack the temporal extent of tree rings necessary to assess how inter-annual variability of C fluxes affects tree radial growth.

Recently, several studies have shown that time series analysis of xylem anatomical traits in tree rings can improve our understanding of terrestrial C fluxes and forest responses to future climate change (Zuidema *et al* 2018, Friend *et al* 2019, Kannenberg *et al* 2020, Puchi *et al* 2020, Babst *et al* 2021). Wood anatomical traits can provide valuable information on tree functioning and indirect evidences of wood formation processes (Fonti *et al* 2010, Carrer *et al* 2017, De Micco *et al* 2019). When wood anatomical variations are assessed at the intra-ring level, intra-seasonal climate influence on tree radial growth can be retrieved, overcoming the typical annual resolution of TRW analysis (Castagneri *et al* 2017, Belokopytova *et al* 2019, Pérez-de-Lis *et al* 2022). Since both ecosystem productivity and wood formation patterns vary considerably over the growing season, and show different sensitivity to seasonal climate (Deslauriers *et al* 2017, Xu *et al* 2020, Krejza *et al* 2022), long time-series analysis at intra-annual scale could disclose the complex relationships between environmental variability, C fluxes and biomass accumulation in wood which are obscured by annually resolved analyses.

This study aims to improve our understanding of the link between C uptake and above-ground woody biomass growth, by investigating the connections between climate variability, ecosystem—atmosphere C fluxes and xylem biomass in an eastern white pine (*Pinus strobus* L.) forest over a 16 year period (2003–2018). We explored the use of quantitative wood anatomy to assess woody biomass accumulation at intra-annual scale, in relation to the temporal variability of climate and GPP. The specific objectives of the study were to (a) compare the inter-annual variability of C uptake and woody biomass through wood anatomical

proxies (cell wall area (CWA) of individual cells and accumulated for the EW and LW); (b) assess how daily mean temperature, precipitation and vapor pressure deficit (VPD) influence C uptake and woody biomass at inter- and intra-annual scale and at intra-ring level.

## 2. Materials and methods

### 2.1. Study site and climate

The study area consists of an eastern white pine (*Pinus strobus* L.) stand planted in 1939 (known as TP39 or CA-TP4 in global Fluxnet) located near Turkey Point, Ontario, Canada (42° 42'N, −80° 21'E, 184 m a.s.l., figure 1; Arain 2018). Tree species at the site include 82% white pine with the average tree height of 23.4 m and tree density of 321 trees ha<sup>−1</sup>. The soil in the region is well drained with low water holding capacity and composed of ~98% sand and is classified in the Canadian System of Soil Classification as lacustrine- derived Brunisolic grey-brown luvisol. The mean water table depth ranged from about 6.8 m in winter-spring to about 6.2 in summer-autumn over the study period from 2003 to 2018. For more site details see Arain *et al* (2022).

The climate in the region is characterized as humid temperate with cold winters and warm summers. Long-term meteorological data used in this study were obtained from the Delhi weather station (42.871 °N, 80.550 °W; <http://climate.weather.gc.ca/>, Ministry of Environment and Climate Change Canada; Environment Canada 2017) located approximately 24 km northwest of the TP39 site. Mean annual temperature was 8.1 °C for the period 1970–2018 with mean daily maximum temperature of 21.0 °C for the hottest month (July) and mean minimum daily temperature of −5.4 °C in January. Mean annual precipitation was 967 mm for the same period, distributed quite evenly over the year (figure 1(b)). In addition, relative humidity (RH) was obtained from Delhi station, to calculate VPD (hPa) based on the Tetens formula (Tetens 1930).

### 2.2. EC flux measurements

Half-hourly fluxes of energy, water vapor and C dioxide (CO<sub>2</sub>) were measured from 2003 to 2018 using a closed path EC system throughout the study period (Arain *et al* 2022). Air was sampled at 20 Hz on top of the tower and fluxes were calculated at half-hourly intervals. Net ecosystem CO<sub>2</sub> exchange was calculated by adding CO<sub>2</sub> flux and the rate of CO<sub>2</sub> storage change in the air column below the EC sensors. Ecosystem respiration (RE) was calculated as a non-linear relationship between nighttime NEP and soil temperature at 5 cm depth and volumetric water content in the 0–30 cm soil layer. GPP was calculated by summing NEP and RE values.

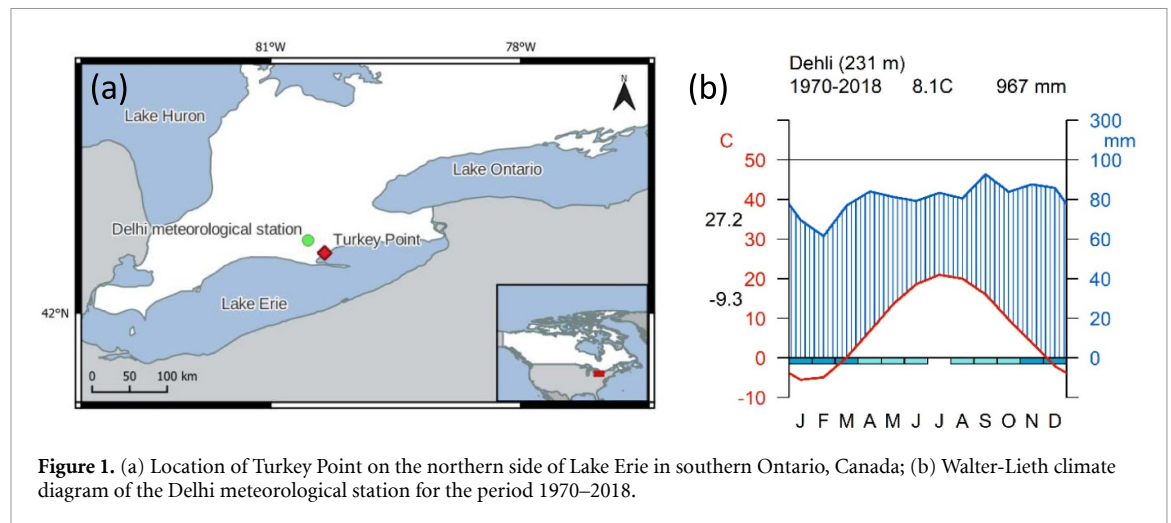
Meteorological variables measured at the EC tower included: air temperature and RH, wind speed and direction and soil temperature and soil moisture at several depths. Precipitation was measured using an all-season, heated, tipping-bucket rain gauge (model 52 202, R.M. Young Co.) from 2003–2007 and using an accumulation rain gauge (model T200B, Geonor Inc.) thereafter (figure S1) (see Arain *et al* 2022 for more details).

### 2.3. Tree ring sample collection and wood anatomical measurement

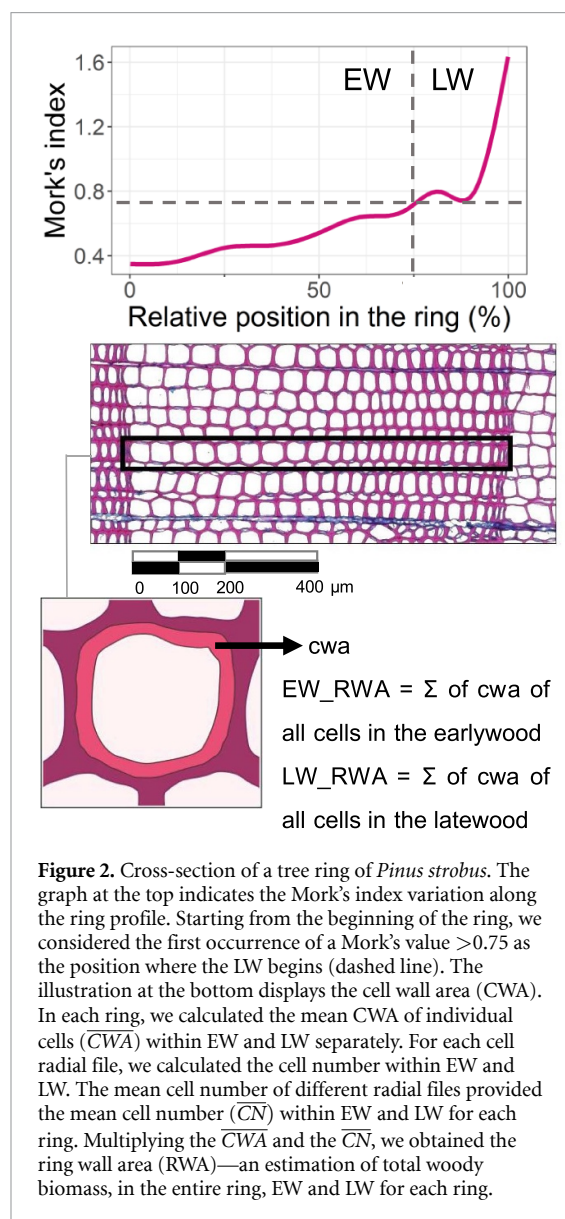
In 2019, within the EC tower footprint, 12 trees were selected and two cores per tree were extracted at 1.3 m from ground level using an increment borer of 5 mm diameter. Stem diameter at breast height of sampled white pine trees was 44.8 ± 6.0 cm, and tree height was 24.0 ± 1.7 m. In the laboratory, all cores were sanded with increasingly finer sandpaper to evidence tree ring boundaries. Then, TRW were measured to the nearest 0.01 mm using TsapWin (Rinntech, Heidelberg, Germany), cross-dated using standard dendrochronological methods, and checked for dating and measurement errors with COFECHA (Stokes and Smiley 1968, Holmes 1983).

For wood anatomical analysis, one core per tree was split in 3–4 cm long pieces, from which thin (12 µm) cross-sections were obtained with a rotary microtome (Leica, Heidelberg, Germany). The sections were stained with a solution of safranin (1%) and astrablue (0.5%), and permanently fixed on glass slides using Euparal (Merck, Darmstadt, Germany), and finally digitized using an Axio Scan. Z1 slide scanner (Zeiss, Jena, Germany) with a resolution of 2.27 pixels µm<sup>−1</sup>. Later, the images were processed in ROXAS, an image analysis software, providing measurements of each tracheid including its relative position within the tree ring (von Arx and Carrer 2014). The wood anatomical measurements were performed for the period 1970–2018.

Among several parameters given by the software, we focused on the CWA as a two-dimension measurement of the cell biomass. Each cell was considered in its relative position within the dated annual ring, allowing us to assess intra-ring variability of CWA for rings with different cell number (CN) (Castagneri *et al* 2020). We distinguished EW from LW using a Mork's index of 0.75 (Denne 1989) (figure 2). For EW and LW separately, we calculated the mean CWA ( $\overline{CWA}$ ) in each ring. Then, we used the RAPTOR R package (Peters *et al* 2018) to obtain the CN for each radial file. The mean CN of different radial files provided the mean cell number ( $\overline{CN}$ ) within EW and LW for each ring. Finally, multiplying the  $\overline{CWA}$  and  $\overline{CN}$ , we obtained the ring wall area (RWA)—an estimation of total woody biomass in EW and LW for each ring (figure 2). Since >90% of conifer wood is formed



**Figure 1.** (a) Location of Turkey Point on the northern side of Lake Erie in southern Ontario, Canada; (b) Walter-Lieth climate diagram of the Delhi meteorological station for the period 1970–2018.



**Figure 2.** Cross-section of a tree ring of *Pinus strobus*. The graph at the top indicates the Mork's index variation along the ring profile. Starting from the beginning of the ring, we considered the first occurrence of a Mork's value  $>0.75$  as the position where the LW begins (dashed line). The illustration at the bottom displays the cell wall area (CWA). In each ring, we calculated the mean CWA of individual cells (CWA) within EW and LW separately. For each cell radial file, we calculated the cell number within EW and LW. The mean cell number of different radial files provided the mean cell number ( $\overline{CN}$ ) within EW and LW for each ring. Multiplying the  $\overline{CWA}$  and the  $\overline{CN}$ , we obtained the ring wall area (RWA)—an estimation of total woody biomass, in the entire ring, EW and LW for each ring.

the biomass estimate based on the wood structure was a very reliable estimate of the C amount in tree rings.

#### 2.4. Assessing climate influence on GPP with TRW and wood anatomy

TRW and xylem anatomy series were standardized to remove the typical age/size related trends using a 15 years cubic smoothing spline (Cook and Kairiukstis 1990, Carrer *et al* 2015). By computing the bi-weight robust mean from the detrended individual time series, we built mean chronologies of EW\_CWA, LW\_CWA, EW\_RWA and LW\_RWA from 1970 to 2018 (figures S2 and S3). Analyses were performed using the R package dplR (Bunn 2008).

We checked for temporal trends in the EC flux and climate data. Daily GPP and RE presented a positive trend (figure S4) and were detrended using the same spline function used for anatomical chronologies, in order to focus on interannual variability. NEP did not present any trend (figure S4). Mean temperature and VPD showed a positive trend, and were detrended, while precipitation did not present a significant trend (data not shown).

We calculated Pearson's correlations between wood anatomical mean chronologies, GPP, RE and NEP, and mean temperature, VPD and precipitation series from the day of the year (DOY) 60 (start of March) to 334 (end of November), for the times of the year when GPP was above 0. Using a daily\_response function in the DendroTools package (Jevšenak and Levanič 2018) we detected the window of maximum correlation coefficient of consecutive days. The correlation analysis was conducted for the periods covered by wood anatomical chronologies (1970–2018), and the EC data (2003–2018), respectively. Finally, to determine the relationship between C fluxes and woody biomass at inter- and intra-annual scale, we correlated EW\_CWA, LW\_CWA, EW\_RWA and LW\_RWA with daily GPP, RE and NEP using the daily\_response function for the period 2003–2018. We found that correlations with GPP were strongest,

of tracheids (Carlquist 1988, Chave *et al* 2009), and the C concentration in wood of temperate conifers is quite constant ( $50.1\% \pm 0.4$  (s.e.)) (Martin *et al* 2018),

therefore only analyses on GPP were further analyzed and presented.

### 3. Results

#### 3.1. Climate association with GPP and woody biomass

Mean temperature and especially VPD were strongly positively correlated with GPP in early-spring and negatively during summer (figure 3, table S1). GPP was positively and significantly correlated with precipitation during mid spring until early June.

CWA and RWA chronologies (1970–2018) were positively correlated with each other in both EW (Pearson's  $r = 0.52$ ,  $p < 0.001$ ), and LW (Pearson's  $r = 0.87$ ,  $p < 0.001$ ). Positive correlations were also observed between EW\_CWA and LW\_CWA (Pearson's  $r = 0.50$ ,  $p < 0.001$ ), and between EW\_RWA and LW\_RWA (Pearson's  $r = 0.66$ ,  $p < 0.001$ ) chronologies (figure S3). EW\_CWA was negatively correlated with precipitation in spring and positively in summer, while mean temperature and VPD showed the opposite pattern (figure 4, table S1). LW\_CWA was positively correlated with mid-summer precipitation and to mean temperature during most of the growing season. Correlation with VPD shifted from positive to negative in the mid of the growing season (figure 4, table S1). EW\_RWA was strongly positively correlated with mid-summer precipitation, while mean temperature and VPD showed a positive correlation in spring and shifted to strong negative correlation around May (figure 4, table S1). Conversely, LW\_RWA was strongly positively correlated with mean temperature and VPD during spring and summer (figure 4, table S1).

#### 3.2. Coupled variability of GPP with TRW and woody biomass

TRW and total ring RWA were highly correlated ( $r = 0.94$ ) to each other, but RWA had a higher correlation with annual GPP ( $r = 0.73$ ; figure 5).

CWA and annual GPP showed a steady increase from 2003 to 2018 (figures S3 and S4). Even removing this common trend, woody biomass proxies in EW and LW showed significant positive relationship with GPP during the growing season (figure 6,  $p < 0.01$ ). Specifically, EW\_CWA and GPP were correlated in late spring and summer (10 May to 1 August), while LW\_CWA was strongly correlated to summer GPP (3 July to 3 September). EW\_RWA correlated strongly with GPP during most of the growing season (25 April to 15 September). LW\_RWA presented strong correlation with GPP during the second half of summer (6 August to 15 September; figure 6).

#### 3.3. Intra-annual dynamics of GPP and woody biomass

In general, GPP started to increase from late March, peaked in July and decreased in October (figure 7,

table S3). In most of the early years, GPP showed a bell-shaped curve and the CWA profile was quite flat. However, in the last years GPP showed higher values in August, and CWA showed a gradual increase with a peak at 80%–90% of the ring.

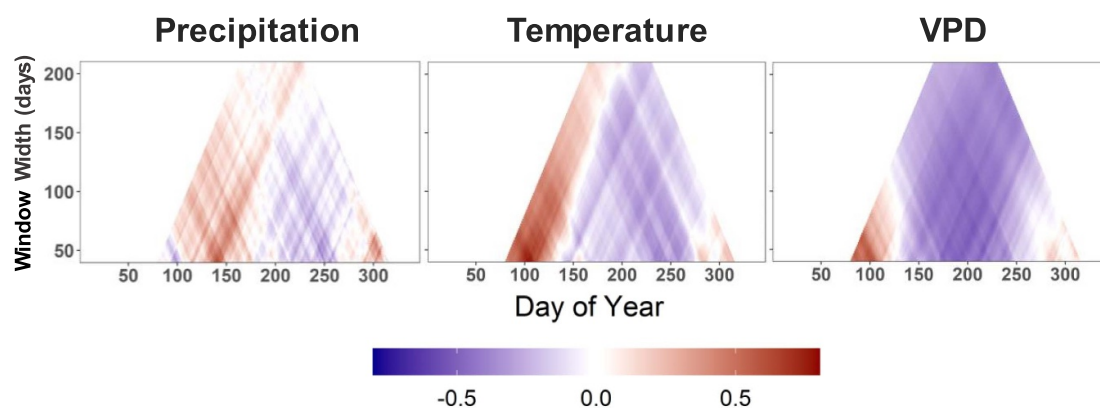
Interestingly, intra-annual variability of GPP and CWA showed common patterns. For example, in 2003, GPP was higher in August compared to 2004 and 2005, and the CWA at the end (around 90%) of the ring was also higher in 2003 compared to the two successive years. Year 2007 experienced drought from May to July—that year GPP was reduced from May to August and CWA in the second part of the ring was lower than in the previous and successive years. The year 2011 was characterized by dry June and July, and the hottest July registered in the 16 years of measurements at the site, which caused an unusual reduction of GPP in July. This was reflected in a strong CWA decrease at 60% of the ring width. Returns to normal temperature and abundant precipitation in August 2011 led to the recovery of GPP and CWA. During the warm and dry spring and summer of 2012, GPP was lower compared to 2013 and 2014 from May to September, and CWA was reduced along the entire ring (figure 7, tables S2 and S3). In 2016, spring and early summer were dry, GPP reduced compared to previous and successive years, and CWA was reduced in the EW.

### 4. Discussion

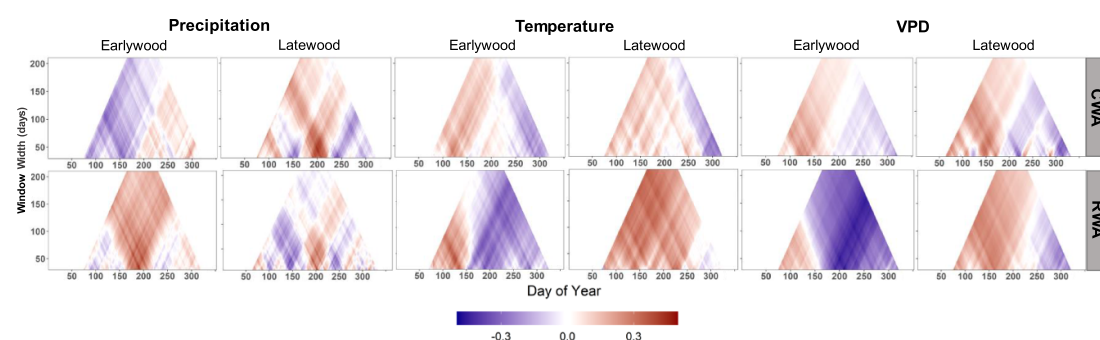
We found strong evidence that C uptake (GPP) and woody biomass (CWA and RWA) were highly coupled at inter- and intra-annual scale in the study site. This study demonstrated the potential of retrospective analysis of wood anatomical traits to improve our understanding of the link between forest C fluxes and woody biomass accumulation. It shows that inter- and intra-annual information, which cannot be provided by traditional tree ring analyses, is critical to disentangle the causes of variable and often weak associations between GPP and woody biomass variability (Cabon *et al* 2022, Kannenberg *et al* 2022).

#### 4.1. Intra-annual analysis on woody biomass and GPP

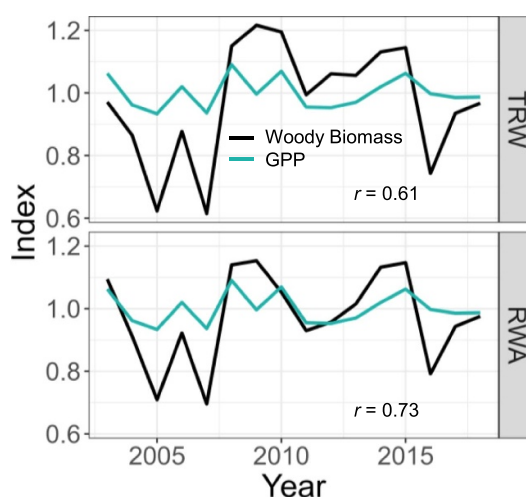
We found strong correlation between TRW and GPP in the current year ( $r = 0.61$ ), similarly to previous study at the site by McKenzie *et al* (2021). Such association was quite high as compared with other studies that have found no or marginal correlations between GPP and ring width (see Cabon *et al* 2022 for an overview). This suggests that C accumulation in wood is mostly source driven (Fatichi *et al* 2014) at our study site. However, by using anatomical proxies of biomass at intra-annual resolution and daily resolved analysis of correlation, we considerably improved correlations up to 0.89 for LW CWA and GPP from 3 July to 3 September.



**Figure 3.** Pearson's running correlations between GPP and daily precipitation, mean temperature and VPD from March to November (Day Of Year 60–334) for the period 2003–2018. The DOY on the *x*-axis represents the starting DOY and the subsequent days of the respective window width, shown on the *y*-axis (time windows of 30–210 d). Negative correlation coefficients are coded in blue and positive in red colors.



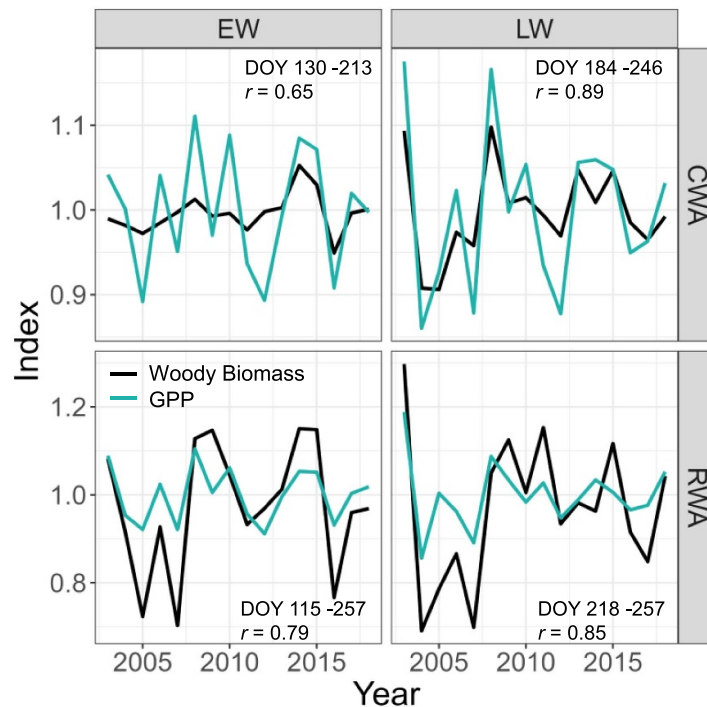
**Figure 4.** Pearson's running correlations between CWA and RWA chronologies in earlywood and latewood and precipitation, mean temperature and VPD from March to November (Day Of Year 60–334) for the period 1970–2018. The DOY on the *x*-axis represents the starting DOY and the subsequent days of the respective window width, shown on the *y*-axis (time windows of 30–210 d). Negative correlation coefficients are coded in blue and positive correlation coefficients in red.



**Figure 5.** Standardized chronologies of TRW and total ring RWA (black lines) and annual GPP (green lines) for the period 2003–2018, and correlations among them (Pearson's *r*, all significant at  $p < 0.05$ ).

Other intra-annual approaches also showed improvement compared to classical ring width analysis. For example, Delpierre *et al* (2016) did not find any correlation between above-ground biomass

and C inputs at annual scale, however at weekly and monthly scale (using dendrometers) significant correlations arose ( $r = 0.55$ ,  $r = 0.75$ , respectively). Using a biometric modeling approach Metsaranta



**Figure 6.** Standardized chronologies of CWA and RWA (black lines) and GPP (green lines) in earlywood (left panels) and latewood (right panels) for the period of the year (DOY interval) that showed maximum correlation (Pearson's  $r$ ). All correlations are significant at  $p < 0.01$ .

*et al* (2021) found a strong and significant correlation between biomass and GPP from May to December in boreal forest of Canada ( $r \geq 0.5$ ,  $p < 0.05$ ). All these studies suggest that the intra-annual analysis can help to clarify and interpret past results obtained at annual resolution, which might be too coarse to assess the link between GPP and wood biomass growth.

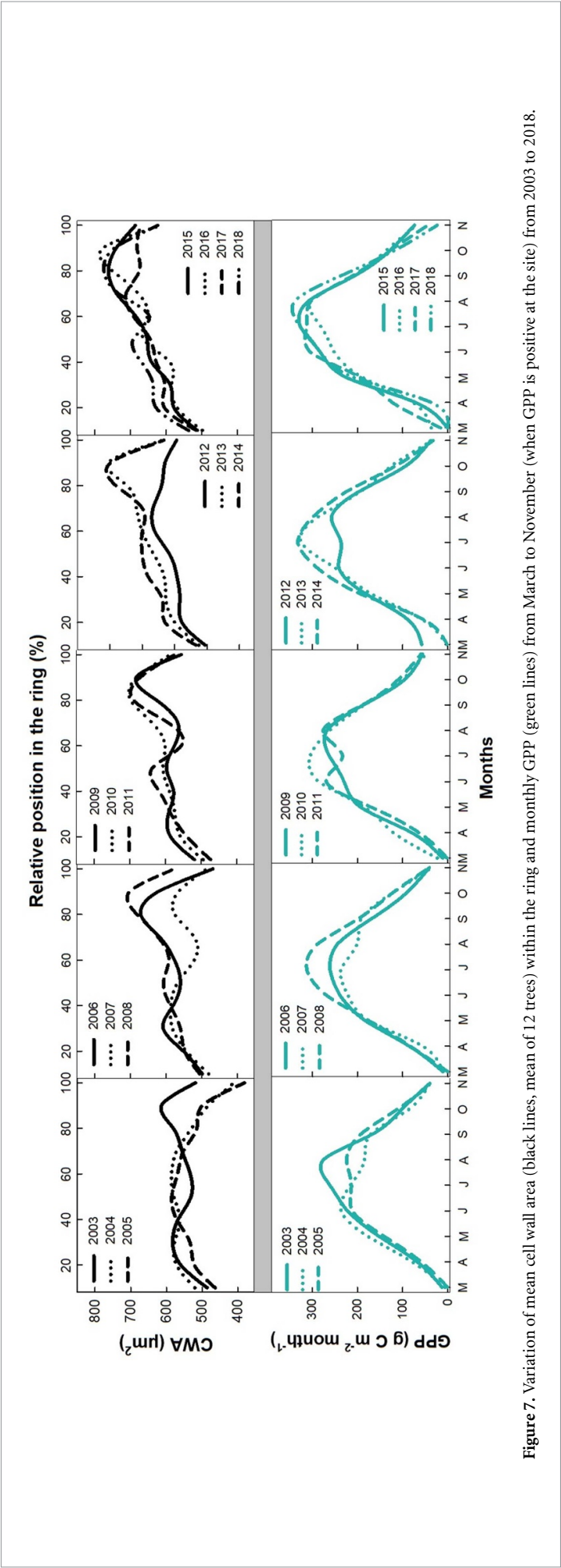
#### 4.2. Climate impacts on C uptake

Our investigation, conducted at intra-annual resolution, showed that GPP was affected by the seasonal course of climate. While warm and wet springs increased GPP, from May onwards high temperature and VPD had a negative effect. The drought sensitivity of EC fluxes observed at the site was exacerbated by well-drained sandy soils with reduced water holding capacity (McLaren *et al* 2008, Beamesderfer *et al* 2020). In conifers, the physiological mechanism to cope with drought is to close the stomata to reduce water loss (isohydric regulation, McDowell *et al* 2008, Green *et al* 2019). As a consequence, it can significantly reduce the carboxylation efficiency (O'Sullivan *et al* 2017), and lower both carboxylation and electron transport rates (e.g. Duarte *et al* 2016, Dusenage *et al* 2019). Moreover, during hot summers GPP can be constrained directly by thermal damage to the photosynthetic system and indirectly due to reduced stomatal conductance and leaf water potential under heat stress (Duarte *et al* 2016, Baldocchi 2020, Smith *et al* 2020).

Over the study period of 16 years, we observed a steady increase of GPP and RE. NEP, as the difference between GPP and RE, was quite constant.  $\text{CO}_2$  fertilization was probably the main cause for the increase in GPP. Recent studies on EC networks on a global scale (Chen *et al* 2020), and with free-air  $\text{CO}_2$  enrichment (FACE) experiments (Walker *et al* 2019, Jiang *et al* 2020) indicated that increased photosynthetic activity in the recent decades was caused by enhanced atmospheric  $\text{CO}_2$  concentration. However, the increase of temperature, VPD and drier soils will reduce future global net ecosystem productivity due to increased RE. Accordingly, we observed a negative influence of summer temperature on interannual variability in GPP. However, warming-induced lengthening of the growing season might increase the annual C uptake, due to an earlier spring phenology and later cessation of photosynthetic activity in autumn observed at the study site (Beamesderfer *et al* 2020).

#### 4.3. Woody biomass variability and its association with seasonal GPP

We found that primary productivity had a strong influence on woody biomass accumulation in EW and LW. We showed that GPP depended on the seasonal climate variability, particularly spring and summer drought, and this finally affected the woody biomass formed in the early and late part of the xylem formation season.



**Figure 7.** Variation of mean cell wall area (black lines, mean of 12 trees) within the ring and monthly GPP (green lines) from March to November (when GPP is positive at the site) from 2003 to 2018.

We found similar patterns of intra-annual variations of GPP and intra-ring variations of cell biomass. We cannot determine with high resolution the timing of cell formation with our retrospective approach, still considering the climate at the site and several studies conducted on conifers in similar climate conditions, we can assume that cell formation starts between April and early May and xylogenesis ends with the LW cell wall thickening phase in late summer to mid-autumn (Rossi *et al* 2008, 2016, Buttò *et al* 2019). We observed that the typical intra-annual pattern of CWA was quite similar to that of GPP. Furthermore, inter-annual variability of EW CWA was associated to spring and early summer GPP, while LW CWA was associated to GPP from July to the beginning of September, suggesting that cell biomass variability was directly related to the C uptake in the season. Finally, the common intra-annual variations of GPP and intra-ring variation of CWA observed in specific years suggests that concurrent heat and drought events can rapidly decrease C sequestration during the wood formation, as observed in a recent study on xylem formation monitoring in Norway spruce (Martínez-Sancho *et al* 2022).

Both GPP and woody biomass proxies presented an increasing trend over the 16 years of data analyzed in our study. Considering tight relationships observed in this stand at the inter- and intra-annual scale, we inferred that the likely causes of the long-term increase of primary production—CO<sub>2</sub> fertilization and the growing season lengthening (Chen *et al* 2020)—eventually affected the cell biomass. According to this, we might therefore expect higher biomass accumulation in the future. However, more frequent and severe droughts and temperature extremes might reduce CO<sub>2</sub> fertilization effects on tree C uptake and accumulation to woody biomass, affecting the stand capacity to sink C (Anderegg *et al* 2015, Green *et al* 2019, McDowell *et al* 2020, Humphrey *et al* 2021). While CO<sub>2</sub> fertilization effects can be expected along the entire year, drought effects might be more temporally limited, affecting biomass accumulation during specific periods, depending on the timing and duration of drought.

## 5. Conclusions

We innovatively analyzed C woody biomass on an intra-annual scale using retrospective analysis of wood anatomical traits and coupled this with GPP derived from EC measurements. This improved the correlation between GPP and biomass estimates compared to the classical ring width analysis and enabled the identification of intra-annual patterns. This approach allowed us to study the basic structural unit behind woody biomass—the cell. We showed that photosynthesis has a strong control over the mechanisms of C accumulation in the woody biomass

(source limitation) at the cell level in white pine at the study site.

The integration of long-term and temporally-detailed estimates of primary productivity at both the local (e.g. EC GPP and NPP) and regional (e.g. satellite-derived NDVI) level with intra-annual information on forest biomass growth (e.g. through xylogenesis monitoring or the intra-ring analysis of wood anatomy) is a promising new line of research. This can help reconcile the difference between C uptake and fixation reported in most previous studies, reducing uncertainties in model projections on the feedback between terrestrial C cycle and climate.

## Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: [10.17190/AMF/1246012](https://doi.org/10.17190/AMF/1246012).

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