Examining the relationship between leaf-mass-per-area and tree age in deciduous trees

Introduction:

The leaf-mass-per-area (LMA) is a morphological trait associated with a variety of plant functions, including photosynthesis and biomass allocation (Poorter *et al*., 2006; de la Riva *et al*., 2016). The physiological determinant of LMA is mesophyll thickness, which concomitantly affects all the aforementioned plant functions. Studies highlight a distinct difference between gymnosperm and angiosperm mesophyll thickness - angiosperm trees exhibit decreased mesophyll thickness and consequently, decreased LMA compared to gymnosperms (Poorter *et al*., 2009). Kröber *et al*. (2014) later showed that gymnosperm trees also exhibit higher mass-based photosynthesis rates because of their higher mesophyll thickness and LMA. While mesophyll thickness defines the LMA of plants, the variation in LMA is driven by a variety of other factors: it varies interspecifically (Shipley, 1995), temporally and spatially (Jurik, 1986), and with light intensity (Martin and Thomas, 2013), among others. Defining the drivers of said variation is of central importance to understanding its effects on the metabolic processes in trees, as well as effectively predicting their responses to climate change (Alberto *et al*., 2013).

The strength and magnitude of the variation in LMA are poorly understood and complex due to the number of factors that drive it (Poorter *et al*., 2006; Hassiotou *et al*., 2010; de la Riva *et al*., 2016). Nevertheless, certain relationships have been observed repeatedly, including a positive correlation between LMA and tree size (Martin and Thomas, 2013). Tree size, often derived from diameter at breast height (DBH), is a common metric for estimating tree growth rates, above-ground biomass, and tree age (Villar *et al*., 2013). The latter can be estimated using a non-destructive method: linear equations derived from empirical data (Sumida *et al*., 2013; Ellis *et al*., 2015). While tree size is the variable most often used in examining tree LMA trends - tree size and LMA have been shown to have a positive relationship (Martin and Thomas, 2013), studies also link tree age, as a derivative of tree size) and LMA in trees. Lichtenthaler *et al*. (2007) and Hassiotou *et al*. (2010) showed that tree age and light availability impact LMA in deciduous tree species, however, it remains unclear whether the effect on LMA is the interaction between these two factors.

Souza *et al*. (2021) have previously shown that light availability to each leaf is dependent on its location within the canopy. This study thus aims to eliminate light availability by canopy position as a confounding factor in determining the effect of tree age on LMA. We examined the effect of tree age (derived from DBH) on the lower-canopy leaves of four angiosperm tree species. We expect tree age and LMA to also have a positive relationship within deciduous species, similar to that between tree size and LMA. We also expect to find interspecific variation in LMA, as previously shown by Shipley (1995).

Methods:

<u>Study site and conditions:</u>

The study site is located within the Central Highlands, Scotland (see Figure 1a). The ecology of the region is characterised by the metamorphic rock geology (Troll *et al*., 2009) and its specific climatic conditions - the mean annual precipitation of the region is 1427 mm and the mean annual temperature ranges from 5.2℃ to 12.4℃ (Met Office, 2020). For our study, 40 trees were sampled within a 3.51 ha area of the Central Highlands on 08.09.2022 (see Figure 1b).

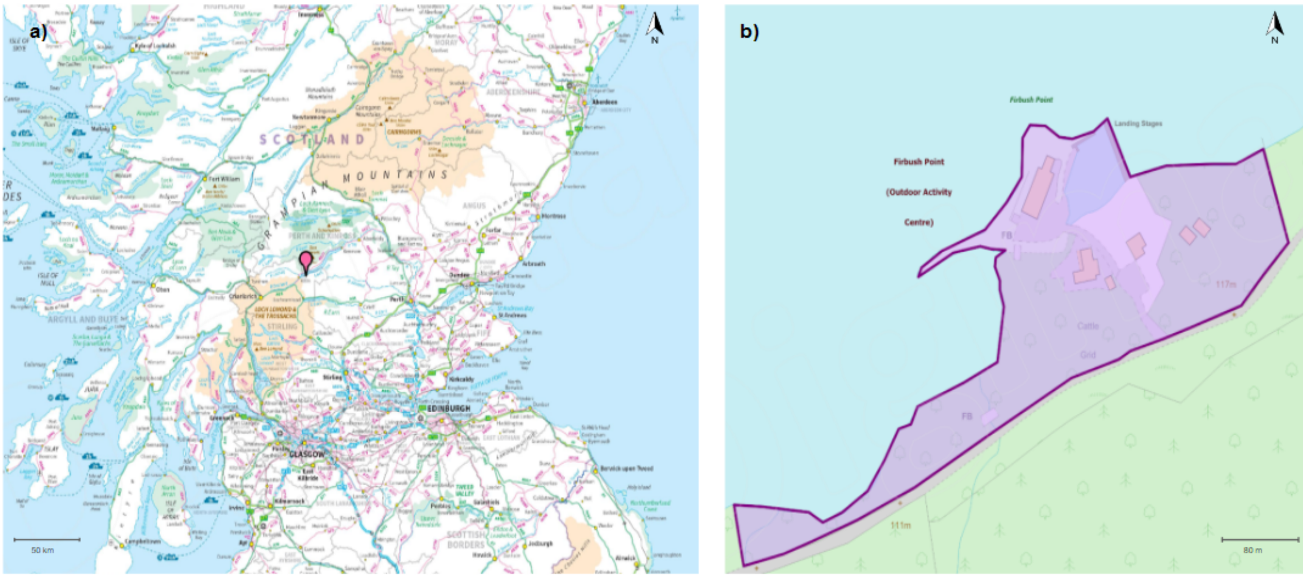

Figure 1a shows the location of the study site in the Central Highlands, Scotland (NN 602 337). **Figure 1b** shows the sampling area.

<u>Data collection:</u>

We selected 10 trees of each species - *Alnus glutinosa, Betula pendula, Quercus petraea*, and *Sorbus aucuparia* - and measured 3 variables: tree DBH, leaf mass, and leaf area for each tree. The trees were selected randomly, however, the distance between the two canopies had to exceed 3 m to avoid any overlap.

<u>DBH and tree age estimation</u>:

The DBH (in cm) was measured at 1.3 m using DBH tape, taken at two points perpendicular to each other on each tree to account for any structural variation in the trunk. The mean DBH of each tree was obtained from the two measurements and used to calculate the age of each tree using linear equations derived by Ellis *et al.* (2015) - see Table 2.

Species	Age equation
A. glutinosa	y = 0.753x + 37.09
B. pendula	y = 0.919x + 34.2
S. aucuparia	y = 0.991x + 10.67
Q. petraea	y = 0.909x + 34.29

Table 2 shows the DBH-to-age equations for each tree species where x is the tree DBH (in cm) and y is the tree age (in years) (from Ellis *et al*., 2015).

LMA measurement:

We collected 16 leaves from the lower canopy (up to 3m from the ground) of each tree, 4 per cardinal point to account for any variation in solar radiation. The area of four randomly selected leaves (including the petioles) was calculated (in cm^2) from photographs using ImageJ (version 1.53). The mean leaf area for each tree was extrapolated from these measurements and converted from cm^2 to m^2. The 16 leaves from each tree were then dried in an oven at 60°C for 72 hours, after which the mean leaf dry mass per tree was measured (in g; including petioles). We used $LMA = \frac{M}{A}$ where M is the mean leaf dry mass (in g), and A is the mean leaf area (in m^2) to calculate the mean LMA for each tree.

Data analysis:

R.studio (version 4.1.3) was used to perform multiple ANOVAs on our data.

Results:

We found no relationship between age and LMA in deciduous tree species (ANOVA, $F_{1,38} = 0.084$, $R^2 = 0.002$, $p > 0.05$, $n = 40$; see Figure 2a) and no relationship between LMA and tree age within the deciduous trees species (ANOVA, for *A. glutinosa, B. pendula, Q. petraea,* and *S. aucuparia*, respectively: $F_{1,8}$ = 0.026, 0.027, 0.110, 0.063, R^2 = 0.003, 0.003, 0.014, 0.008; $p > 0.05$, $n = 10$ for all; see Figure 2b).

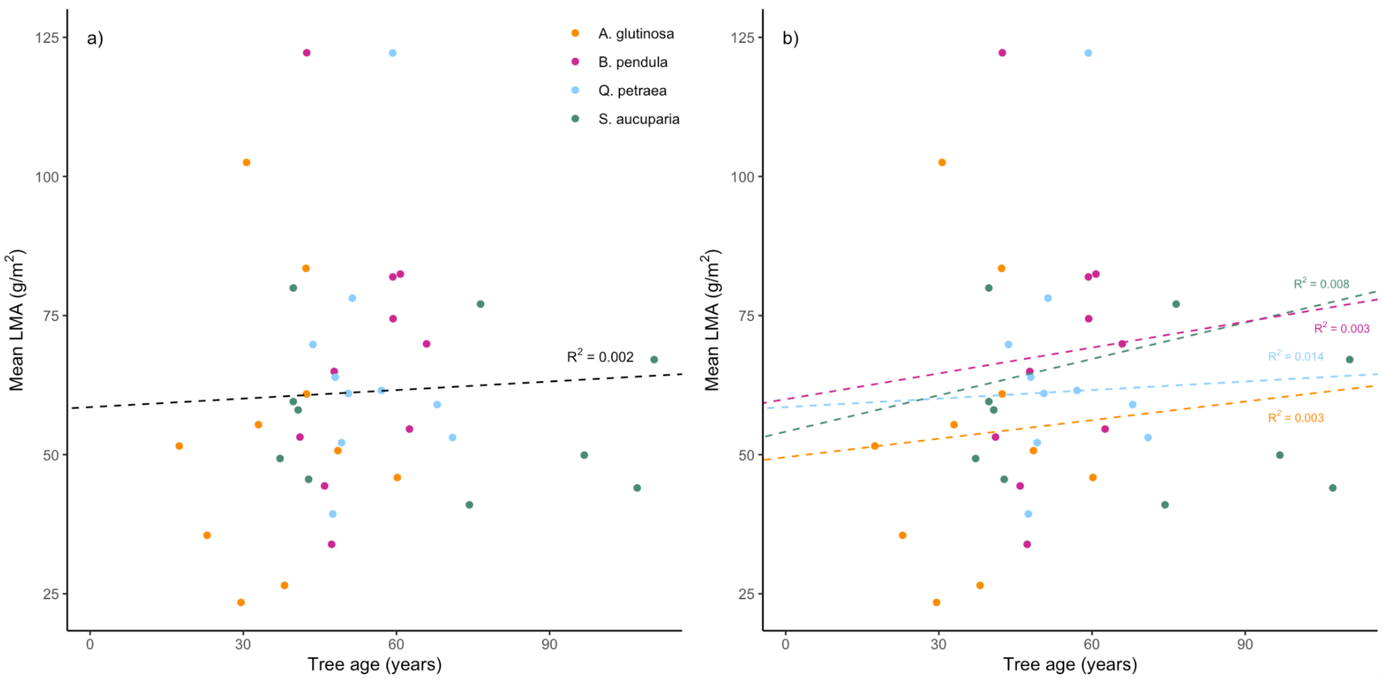

Figure 2a shows no effect of tree age on mean LMA (n=40); **Figure 2b** shows no effect of tree age on LMA within the four different species (n=10). The dots represent raw data, the dotted lines represent the regression lines fit to the data; shown with respective R^2 values.

We also found no significant interspecific variation in LMA (ANOVA, $F_{1, 38}$ = 1.028, $p > 0.05$, n = 40, see Figure 3).

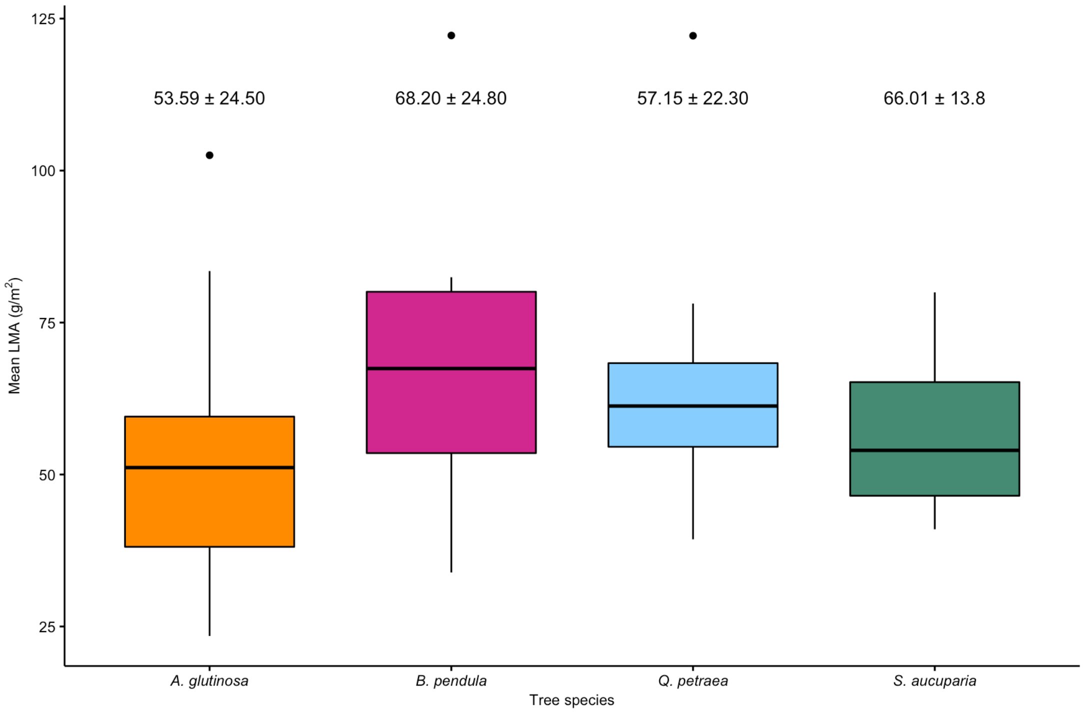

Figure 3 shows the distribution of LMA values for each of the four tree species (n = 40). The boxplots show the minimum, first quartile, median, third quartile, and maximum LMA values for each species; the dots show outliers. The text shows the mean LMA ± the standard deviation (in g/m²).

Discussion:

<u>Study purpose and findings</u>:

Leaf-mass-per-area is an important leaf morphological trait that influences a variety of plant functions and is itself affected by a multitude of factors. This study evaluated the relationship between tree age (derived from tree size) and LMA in deciduous trees: we found no such relationship between or within four deciduous tree species (*A. glutinosa, B. pendula, Q. petraea,* and *S. aucuparia*).

<u>Study limitations</u>:

As we only investigated lower-canopy leaves due to time constraints, assessing the effect of leaf position in the canopy was beyond the scope of this study. Previous studies have shown an effect of light availability and tree age on LMA (Lichtenthaler *et al*., 2007; Hassiotou *et al*., 2010) - we hypothesise that light availability due to canopy position and tree age interact to drive LMA variation. To evaluate this relationship, this study should be repeated with leaf position within the canopy as another variable in evaluating the effect of tree age on tree LMA in deciduous tree species. Including canopy position in future studies might also help explain why we found no interspecific variation in the LMA of deciduous trees - it has been shown LMA varies with canopy height (Ellsworth and Reich, 1993).

Another variable to consider in future studies is the petiole - our measurements included the petioles in our mass and area measurements. Li *et al*. (2008) showed that petiole mass is positively correlated with leaf mass and area - we, therefore, expect the petiole-to-mass and petiole-to-area ratios of the compound *S. aucuparia* leaves to differ from the simple leaves of the other three species. The study should be repeated with leaf morphology and composition as an additional factor - by the exclusion of the petioles in the area and mass measurements.

Impact and future implications:

Defining the drivers of variation in LMA is essential to our understanding of their metabolic processes such as photosynthesis and biomass allocation. We expected tree age alone to influence the LMA of deciduous trees and while our study shows no such relationship and no interspecific variation in LMA, the constraints of the study should not be ignored - it is possible the effect is due to multiple factors. Further research is needed to better understand the drivers of LMA variation and their interactions, specifically in evaluating plant phenotypic plasticity related to unprecedented rates of climate change (Alberto *et al*., 2013; Buraczyk *et al*., 2022). It is essential to climate change mitigation strategies (namely reforestation and existing forest preservation) to understand how trees will respond to changes in the environment, with LMA being one of the central traits dictating carbon fixation and sequestration rates through photosynthesis and biomass allocation (Bussotti *et al.*, 2015).

Conclusions:

LMA is a morphological trait determined by leaf mesophyll thickness and impacts many leaf metabolic processes, including photosynthesis and respiration. It is affected by a multitude of factors, including tree size. This study aimed to determine whether a relationship exists between LMA and tree age (as a derivative of tree size) - we found no such relationship in the lower canopies of four deciduous tree species (*A. glutinosa, B. pendula, Q. petraea,* and *S. aucuparia*). Future studies should thus account for canopy position and the presence of petioles to determine whether the previously shown variation in LMA is caused by an interaction of multiple factors.

References:

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R. and Savolainen, O. (2013). Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology*, 19(6), pp.1645–1661.

Buraczyk, W., Tulik, M., Konecka, A., Szeligowski, H., Czacharowski, M. and Będkowski, M. (2022). Does leaf mass per area (LMA) discriminate natural pine populations of different origins? *European Journal of Forest Research*.

Bussotti, F., Pollastrini, M., Holland, V. and Brüggemann, W. (2015). Functional traits and adaptive capacity of European forests to climate change. *Environmental and Experimental Botany*, 111, pp.91–113.

de la Riva, E.G., Olmo, M., Poorter, H., Ubera, J.L. and Villar, R. (2016). Leaf Mass per Area (LMA) and Its Relationship with Leaf Structure and Anatomy in 34 Mediterranean Woody Species along a Water Availability Gradient. *PLOS ONE*, 11(2), p.e0148788.

Ellis, C., Eaton, S., Theodoropoulos, M. and Elliott, K. (2015). *Epiphyte Communities and Indicator Species An Ecological Guide for Scotland's Woodlands*

Ellsworth, D.S. and Reich, P.B. (1993). Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia*, 96(2), pp.169–178.

Hassiotou, F., Renton, M., Ludwig, M., Evans, J.R. and Veneklaas, E.J. (2010). Photosynthesis at an extreme end of the leaf trait spectrum: how does it relate to high leaf dry mass per area and associated structural parameters? *Journal of Experimental Botany*, 61(11), pp.3015–3028.

Jurik, T.W. (1986). TEMPORAL AND SPATIAL PATTERNS OF SPECIFIC LEAF WEIGHT IN SUCCESSIONAL NORTHERN HARDWOOD TREE SPECIES. *American Journal of Botany*, 73(8), pp.1083–1092.

Kröber, W., Heklau, H. and Bruelheide, H. (2014). Leaf morphology of 40 evergreen and deciduous broadleaved subtropical tree species and relationships to functional ecophysiological traits. *Plant Biology*, 17(2), pp.373–383.

Li, G., Yang, D. and Sun, S. (2008). Allometric relationships between lamina area, lamina mass and petiole mass of 93 temperate woody species vary with leaf habit, leaf form and altitude. *Functional Ecology*, 22(4), pp.557–564.

Lichtenthaler, H.K., Ač, A., Marek, M.V., Kalina, J. and Urban, O. (2007). Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry*, 45(8), pp.577–588.

Martin, A.R. and Thomas, S.C. (2013). Size-dependent changes in leaf and wood chemical traits in two Caribbean rainforest trees. *Tree Physiology*, 33(12), pp.1338–1353.

Met Office. (2020). *UK climate averages*. [online] Available at: https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gfj1d6wgf [Accessed 8/11/2022].

Poorter, L. and Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, 87(7), pp.1733–1743.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. and Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, 182(3), pp.565–588.

Shipley, B. (1995). Structured Interspecific Determinants of Specific Leaf Area in 34 Species of Herbaceous Angiosperms. *Functional Ecology*, 9(2), p.312.

Sumida, A., Miyaura, T. and Torii, H. (2013). Relationships of tree height and diameter at breast height revisited: analyses of stem growth using 20-year data of an even-aged Chamaecyparis obtusa stand. *Tree Physiology*, 33(1), pp.106–118.

Troll, V.R., Emeleus, C.H., Nicoll, G.R., Mattsson, T., Ellam, R.M., Donaldson, C.H. and Harris, C. (2019). A large explosive silicic eruption in the British Palaeogene Igneous Province. *Scientific Reports*, 9(1).

Villar, R., Ruiz-Robleto, J., Ubera, J.L. and Poorter, H. (2013). Exploring variation in leaf mass per area (LMA) from leaf to cell: An anatomical analysis of 26 woody species. *American Journal of Botany*, 100(10), pp.1969–1980.