

1 **Effects of two contrasting canopy manipulations on growth**  
2 **and water use of London plane (*Platanus x acerifolia*) trees**

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30

31 **Abstract**

32 *Aims* Two contrasting canopy manipulations were compared to unpruned controls on  
33 London plane trees, to determine the effects on canopy regrowth, soil and leaf water  
34 relations.

35 *Methods* 'Canopy reduction', was achieved by removing the outer 30% length of all major  
36 branches and 'canopy thinning', by removing 30% of lateral branches arising from major  
37 branches.

38 *Results* Total canopy leaf areas recovered within two and three years of pruning for the  
39 canopy-thinned and reduced trees respectively. Canopy reduction increased mean leaf size,  
40 nitrogen concentration, canopy leaf area density and conserved soil moisture for up to 3  
41 years, whereas canopy thinning had no effects. Another experiment compared more severe  
42 canopy reduction to unpruned trees. This produced a similar growth response to the  
43 previous experiment, but soil moisture was conserved nearer to the trunk. Analysis of <sup>13</sup>C  
44 and <sup>18</sup>O signals along with leaf water relations and soil moisture data suggested that lower  
45 boundary layer conductance within the canopy-reduced trees restricted tree water use,  
46 whereas for the canopy-thinned trees the opposite occurred.

47 *Conclusions* Only canopy reduction conserved soil moisture and this was due to a  
48 combination of reduced total canopy leaf area and structural changes in canopy  
49 architecture.

50 *Keywords:* leaf area; pruning; soil moisture; stable isotopes; subsidence

51

52 **Introduction**

53

54 Tree canopy growth and development is functionally linked to that of root growth and the  
55 exploitation of belowground resources (Comas and Eissenstat 2004, Misson et al. 2006).

56 Variation in the ability of roots to exploit and capture water is well documented as a  
57 determinate of growth and much is known about how canopy water use is anatomically and  
58 hydraulically linked to the control of factors such as canopy leaf area and its transpirational  
59 demand (Whitehead 1998). Restriction of root growth reduces canopy growth and water  
60 use, while reducing the amount of canopy leaf area (CLA) typically may reduce tree water  
61 use. Understanding the relationships between tree water use, canopy leaf area and root  
62 function are used to predict tree growth (Dawson 1996, Wullschleger et al. 1998). It is  
63 particularly important to understand these relationships when considering ways to influence  
64 and control the development and growth of trees in urban as opposed to forest  
65 environments.

66 Trees have large positive impacts on aspects of the urban environment, through  
67 alterations in energy exchange and the provision of ecosystems services. Much needs to be  
68 achieved to ensure their continued presence does not cause unsustainable, or local  
69 depletion of water resources and negative impacts on urban infrastructure above and  
70 belowground (Gill et al. 2007). These impacts can appear as structural damage to low-rise  
71 buildings which is frequently associated with the close proximity of trees. Cutler and  
72 Richardson (1997) for example, surveyed tree related building damage and found for  
73 *Platanus* species no damage cases occurred for trees planted >15 m from buildings, 10%  
74 damage cases occurred at 10 - 15 m, and 90% of damage cases occurred at <10 m with 50%  
75 at <5.5 m. This indicates that damage cases occurred disproportionately as the trees get  
76 nearer to the building. . Trees can extract water from below the foundations causing some  
77 clay subsoils to shrink, ultimately leading to foundation failure and cracks in superstructures  
78 (Biddle 1983, Driscoll 1983, Crilly 2001). In areas with swelling clay soils, in the UK, tree  
79 roots are claimed to have an effect on subsidence incidents in 73% of cases (Loss Prevention  
80 Council 1995). The cost of repairing the damage caused by the failure of domestic house  
81 foundations, due to subsidence, has been and will likely continue to be considerable

82 (Doornkamp 1993). There is potential for saving on remedial costs, in the UK by reducing  
83 the need for rectification work, which averaged £237 million per annum between 2000 and  
84 2010 ,. Expected climate change impacts in the urban environment can only increase these  
85 costs (Sanders and Phillipson 2003). Currently, no methods exist that reliably predict which  
86 trees may cause damage and not all trees near buildings are implicated. Decreasing water  
87 uptake may lessen subsidence risk by conserving soil moisture and reducing clay subsoil  
88 shrinkage. Reducing canopy leaf area by pruning may lessen water uptake and cyclical  
89 pruning is recommended in a risk limitation strategy developed by the London Tree Officers  
90 Association (1995). Tree pruning is perceived as a potentially effective control measure to  
91 conserve soil moisture and so prevent felling which is environmentally and aesthetically  
92 undesirable in urban areas. Despite implementation of the practice there is little if any  
93 critical experimental support which validates its usefulness. Equally there is little  
94 quantitative understanding of the likely water saving and the duration of such saving,  
95 particularly with respect to amenity tree growth in urban environments.

96 In this study we use a range of plant physiological approaches to compare water  
97 demand with soil water supply. Specifically, we have compared canopy development with  
98 leaf water relations, gas exchange, and stable isotope ( $^{13}\text{C}$ ,  $^{18}\text{O}$  leaf water) signals. The  
99 carbon isotope discrimination of leaf material is primarily caused by enzymatic fractionation  
100 ( $^{13}\text{CO}_2$  is slower to diffuse and react than  $^{12}\text{CO}_2$ ). Leaf organic material becomes depleted  
101 during photosynthesis by a few parts per mille (‰), and in relation to the internal:external  
102 concentration ratio of  $\text{CO}_2$  ( $C_i/C_o$ ) across the leaf (Farquhar et al. 1989; Seibt et al. 2008). In a  
103 well-coupled canopy, this organic signal also functionally relates to stomatal conductance  
104 ( $g_s$ ), transpiration and water use, although under light-limited conditions, deep within a  
105 forest canopy, isotope discrimination is high when water use is low (Seibt et al. 2008).  
106 Additionally, the isotopic ratio of leaf water is also diagnostic of transpiration rate, as the  
107 lighter  $\text{H}_2^{16}\text{O}$  preferentially evaporates, and the enrichment of residual  $\text{H}_2^{18}\text{O}$  is dependent

108 on leaf temperature and humidity, as well as precipitation inputs (Farquhar and Cernusak  
109 2005; Cernusak and Kahman, 2013).

110 The experiments presented here aim to develop our understanding of how isolated  
111 amenity trees use water, and to determine the extent to which different canopy  
112 manipulation strategies actually reduce tree water use and soil drying. We hypothesise that  
113 the extent to which soil moisture is conserved by canopy manipulations depends  
114 considerably on the type and extent of canopy reduction applied and on the regrowth  
115 potential of the tree. We tested this hypothesis by using mature isolated trees to which two  
116 different pruning treatments were applied. Comparisons were made between unpruned  
117 trees and those which had their canopies 'reduced' or 'thinned'. These two canopy pruning  
118 treatments are used as standard, by the UK arboricultural industry to reduce CLA. 'Canopy  
119 reduction', reduces the volume of the canopy through an overall reduction of tree height  
120 and spread by removing the outer portions of all major branches. 'Canopy thinning', reduces  
121 the number of lateral branches arising from all major branches, but does not change the  
122 original canopy volume, but its leaf area density (LAD). The normal UK arboricultural  
123 standard is to reduce branch length and leaf area by around 30% (BS 3998:2010, 2010) for  
124 the two methods respectively. We believe that this is the first time this comparison has been  
125 evaluated experimentally. In a subsequent experiment, a more severe canopy reduction  
126 treatment was applied to trees to determine its effect. To quantify tree water use changes  
127 with pruning over time, treatments effects, including changes in soil moisture content were  
128 measured and related to tree canopy architecture changes from measurements of CLA and  
129 LAD and those of leaf development using stable  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic discrimination.

130

131 **Methods**

132

133 Two experiments were carried out consecutively over a four year period. The first experiment  
134 contained twenty-four 28-year-old London plane [*Platanus x acerifolia* (Aiton) Willd. (a hybrid  
135 of the Oriental plane [*P. orientalis*] and American sycamore [*P. occidentalis*)]. The trees were  
136 approximately 20 m in height within a single row spaced at 12 m between trees and were at  
137 East Malling Research (EMR), Kent, UK (51° 17'N, 00° 27'E). The row was adjacent to an  
138 urban road. The soil was predominantly a mixture of the Malling and Barming series, both of  
139 which have approximately 100-200 cm sandy loam topsoil and clay loam overlying clay  
140 subsoil overlying ragstone. Particle size distribution the profile 0 - 23 cm is sand (60 µm - 2  
141 mm) 49%, silt (2 - 60 µm) 30%, clay <2 µm 21%; 23 - 41 cm depth is sand (60 µm - 2 mm)  
142 38%, silt (2 - 60 µm) 35%, clay <2 µm 27%; 41 - 66 cm depth is sand (60 µm - 2 mm) 45%,  
143 silt (2 - 60 µm) 14%, clay <2 µm 41%; 74 - 92 cm depth is sand (60 µm - 2 mm) 24%, silt (2 -  
144 60 µm) 49%, clay <2 µm 27%. The soil is described as well drained with an available water  
145 class that varies between 150 - >200 mm to 1000 mm depth (Fordham and Green 1980).

146 The pruning treatments were applied in late January in year 1 to BS 3998:2010  
147 standard. Canopy reduction (30% height decrease and branch end removal by visual estimate)  
148 was applied to eight trees and canopy thinning (30% lateral branch removal) to another eight  
149 trees, eight control trees remained unpruned. The experimental design was a complete  
150 randomised block with three treatments and four replicates, each treatment plot contained two  
151 trees. The experiment ran for three years (growing seasons). In the second experiment, one of  
152 each pair of trees in each plot in three of the blocks from experiment 1 was severely canopy  
153 reduced (60% original height, 50% diameter decrease) in mid-April in year 4. The design was a  
154 complete randomised block with two treatments and nine replicates.

155

156 *Weather at East Malling*

157

158 In year 1, rainfall in April and May was more than double the 50-year average (Figure 1). The  
159 calculated soil moisture deficit did not start to accumulate until early June and reached a  
160 maximum of 200 mm in August. Although rainfall was exceptionally high during the  
161 preceding winter, it was only 36 mm greater than the 50-year average between April and  
162 September in year 2. Solar radiation levels were greater than year 1. The accumulated soil  
163 moisture deficit reached a maximum of 250 mm. Rainfall was 22 mm greater than the 50-  
164 year average for the April-September period in year 3. Solar radiation levels were similar to  
165 those of year 1 and monthly rainfall was also similar to year 2. The accumulated soil  
166 moisture deficit followed a similar pattern to that found in year 1, but reached a maximum  
167 of only 179 mm. The soil moisture deficit was less severe than in previous years due to a  
168 combination of near average rainfall and a high proportion of dull days that reduced the  
169 potential for evaporation.

170 The weather during year 4 (experiment 2) was much drier than average. Rainfall was  
171 only two-thirds of the 50-year average. The driest months were July, August and September.  
172 Solar radiation levels were generally as great, or greater than, those of previous years and  
173 September was particularly sunny. Average monthly temperatures were 1.5-3.1°C greater than  
174 the 50-year average during June, July and August (data not shown). As a result, the calculated  
175 soil moisture deficit followed a similar pattern to year 2 until the end of July, but it continued to  
176 decline during August and September to a maximum of 368 mm.

177

#### 178 *Soil drying by trees*

179

180 Soil moisture content was measured using a neutron probe which had been calibrated  
181 previously in the experimental soil. Two access tubes per plot were inserted to 1 m depth in  
182 spring of year 1. One tube was 2 m from the tree and the other was mid-way between the two  
183 adjacent trees in an experimental plot i.e. 6 m from each tree for three of the randomised

184 blocks. Measurements were made at 10 cm depth intervals, those specifically at 10 cm depth  
185 were corrected for loss of neutrons from the surfaces according to the method of Grant (1975).  
186 New access tubes were inserted perpendicular to the tree row, at distances of 2 and 6 m from  
187 each tree in the spring of year 4, as necessary for experiment 2. The neutron probe was used to  
188 measure soil moisture at 1-2 month intervals (i.e. 9 June, 21 July, 25 August, 30 November in  
189 year 1; 22 May, 21 June, 31 July, 29 August, 3 October, 6 December in year 2; 18 February, 26  
190 June, 5 August, 3 September, 11 October and 26 November in year 3; 23 January, 2 May, 2 June,  
191 8 July, 11 August, 17 September, 27 October and 12 December in year 4; and 7 January in year  
192 5). Soil moisture content in mm was calculated by multiplying the volumetric moisture  
193 content for each depth by the depth interval (mm). Soil profile moisture content (1 m depth)  
194 was calculated by summing these totals for each depth. All soil moisture deficits were  
195 referenced to total profile moisture content at field capacity, i.e. 30 November year 1 for  
196 experiment 1, and 7 January year 5 for experiment 2. The approach used here is described  
197 in more detail in Cepuder et al (2008).

198

#### 199 *Canopy regrowth*

200

201 Canopy height was measured using a clinometer (Clino Master, Silva Sweden AB, Sweden)  
202 and diameter was calculated by using the average of measurements taken in East-West and  
203 North-South directions across the trunk. Digital hemispherical images were captured (Nikon  
204 CoolPix 950 fitted with fish eye lens, Nikon UK Ltd, Surrey, UK mounted on a self-levelling  
205 platform, Delta-T Devices, Cambridge UK) for each tree in the experiment in May, June, July,  
206 and August year 1, July and August year 2, September year 3 and August year 4. All the  
207 images were taken at the same position below each tree, 1 m from the trunk on the east  
208 side at the base of the canopy.



209 Total canopy leaf area (CLA), leaf area density (LAD) and drip line (periphery of the  
210 canopy base) leaf area index (LAI) were calculated using the HemiView hemispherical digital  
211 image analysis system (Delta-T Devices 1999). This system was developed to determine the  
212 LAI of forest canopies, and was adapted for single tree analysis (Wood 1999). A half-ellipsoid  
213 model was used to represent the 'volumetric shape' of the canopy for all the treatments  
214 applied in experiment 1. A cylinder model was used for the severe pruning treatment  
215 applied in experiment 2 as this gave a better representation of the real modified canopy  
216 shape. Analyses of canopy images of each tree were used to determine LAD, CLA and LAI.

217

218 *Stomatal conductance and xylem water potential ( $\psi_L$ )*

219

220 Stomatal conductance to water vapour ( $g_s$ ) was measured on three fully expanded leaves per  
221 tree using a porometer (EGM-1, PP Systems Harpenden, Hertfordshire, UK) in the region  
222 close to the middle of the leaf, but avoiding the mid-vein. Xylem leaf water potentials ( $\psi_L$ ;  
223 MPa) were measured also on three leaves per tree using a Scholander-type pressure  
224 chamber (SKPM 1400, Skye Instruments Ltd, Powys, UK). Measurements of  $\psi_L$  were made  
225 directly after the  $g_s$  measurements. Leaves were rapidly pressurized (within 15 s) with  
226 nitrogen. Xylem leaf  $\psi_L$  and  $g_s$  were measured during pre-dawn and mid-day measurements in  
227 September during experiment 2. Access to sampling points in the canopy extremes was carried  
228 out by a trained climber.

229

230 *Stable isotopes  $^{13}C$  and  $^{18}O$  discrimination analysis, leaf N concentration and leaf size*

231

232 Dry leaf samples were ground for 15 minutes at 30 Hz in a MM200 Mixer Mill (Glen Creston  
233 Ltd, Middlesex, UK). Sub-samples of 1 mg ( $\pm 0.2$  mg) were weighed into microanalysis tin  
234 cups and crimped. Samples were sent to the Stable Isotope Unit of the James Hutton

235 Institute, Dundee for determination of  $\delta^{13}\text{C}$  and N composition by mass spectrometry.  
236 Samples were measured on an Isotope Ratio Mass Spectrometer (Europa Scientific, Crewe,  
237 UK), calibrated via secondary standards against the Vienna PDB standard as parts per mille  
238 (‰), and were converted to  $\Delta^{13}\text{C}$  assuming a  $\delta^{13}\text{C}$  of  $\text{CO}_2$  in air of -8‰, as defined by  
239 Farquhar et al. (1989).

240 Leaves were collected for experiment 1 in early September year 1, late August year 2  
241 and early September in years 3 and 4 (experiment 2). Eight leaves per tree were collected in  
242 year 1 and three in years 2 and 3 from the WSW side of the canopy at a height of 12 m.  
243 Three trees per treatment were used, except in year 1, when only two unpruned and two  
244 canopy-reduced and one canopy-thinned tree were sampled. In year 4, three leaves were  
245 collected from sun and shade positions separately. Leaf samples used for O isotope analysis  
246 of leaf water were collected on the same dates. In addition, to the sample taken for isotope  
247 analysis, another larger branch sample of 50 leaves was also taken from three trees per  
248 treatment to determine the size of individual leaves in year 2. All leaves were fully expanded  
249 and fourth or fifth from the shoot apex and their leaf areas were measured (Delta-T Devices,  
250 Cambridge, UK).

251 Extraction and analysis of bulk leaf water was conducted according to Wang and  
252 Yakir (1995). An exetainer containing the leaf sample was attached to the vacuum line, via a  
253 19 G needle (1.5 mm), and frozen in liquid N for 2 minutes so that the exetainer could be  
254 evacuated to a pressure of 3 Pa. The system was then isolated under vacuum from the main  
255 manifold. The exetainer was heated with a water bath at 80°C and cold chilled with liquid N  
256 to freeze the water derived from the sample. An in-line vacuum gauge was used to ensure  
257 that all of the leaf water was removed. After distillation the extracted water was stored in  
258 glass vials and activated charcoal was added to remove any volatile organic contaminants.  
259 Subsamples 0.5 cm<sup>3</sup> were taken of extracted water and placed in glass tubes, when  $\text{CO}_2$  of  
260 known  $\delta^{18}\text{O}$  added at approximately 60 kPa to the tube via the preline manifold. Tubes

261 were sealed with a gas torch and left to equilibrate for at least 3 days for the  $^{18}\text{O}$  signal to  
262 transfer to the  $\text{CO}_2$ . At least one standard (i.e. water with a known  $\delta^{18}\text{O}$ ) and one blank tube  
263 containing no water were included in each batch. Details of the process are given in Epstein  
264 and Mayeda (1953). After equilibration,  $\text{CO}_2$  samples were purified by condensing in a liquid  
265 N trap and acetone chilled to  $-80^\circ\text{C}$ , releasing the  $\text{CO}_2$  while continuing to trap any water.  
266 Frozen water vapour was transferred to a mass spectrometer analysis (VG 903, modified by  
267 ProVac, Crewe, UK) vial, with the separated  $\text{CO}_2$  analysed for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}_{\text{PDB}}$ . The  $\delta^{18}\text{O}_{\text{PDB}}$   
268 was converted to  $\delta^{18}\text{O}_{\text{VSMOW}}$  and corrected for molecular ratios of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in the  
269 samples. The  $\delta^{18}\text{O}$  value was also corrected for the  $\delta^{18}\text{O}$  of the  $\text{CO}_2$  used during equilibration.

270

#### 271 *Calculated soil moisture deficit*

272

273 Data were collected throughout the period of the two experiments from a UK  
274 Meteorological Office approved automatic weather station  $\sim 700$  m east of the experimental  
275 trees. Rainfall (mm) (and the last 50 year average rainfall) and total solar radiation ( $\text{mW cm}^{-2}$ )  
276 data were collected for each year. Calculated soil moisture deficits were calculated by  
277 subtracting measured rainfall from the Penman's calculated daily evaporation (Berry 1964).

278

#### 279 *Statistical analysis*

280

281 Statistical analyses for canopy growth, isotope data and soil moisture deficits were carried out  
282 using ANOVA with GenStat for Windows (VSN International 2011). The soil moisture deficits  
283 data sets for experiments 1 and 2 both involved repeated measures for the same trees, in  
284 the case of experiment 2, for various sampling positions, and for experiment 1 for sampling  
285 position and for year. The difference in maximum deficit between the pruned and unpruned  
286 trees was calculated by subtracting the average deficit for each year for the unpruned trees

287 from the maximum deficit for the individual trees in the pruning treatments in each year.  
288 This enabled pruning treatment effects to be differentiated from effects caused by year-to-  
289 year variation in climate, particularly rainfall. Mixed effect models (Zuur et al. 2009) with  
290 random intercept components for statistical inference were used. In the case of experiment  
291 1 the fixed effects in the model were year and sampling position, in the case of experiment 2  
292 just the sampling position. Approximate standard error estimates were derived from the  
293 mixed effect models. A Friedman test was applied to the means of the maximum difference  
294 from unpruned trees for soil moisture deficits for the 2 m and 6 m distance data across all  
295 years in experiment 1. Wilcoxon signed-rank tests were applied to the means of the  
296 maximum difference of soil moisture deficits from unpruned trees for the 2 m and 6 m  
297 sampling positions in experiment 2. These analyses of differences in maximum deficits were  
298 carried out using R (R Development Core Team 2008).

299

## 300 **Results**

301

### 302 *Canopy manipulation - effects on soil moisture*

303

304 The soil moisture deficit increased rapidly during summer following pruning (year 1)  
305 reaching a maximum in August (Figure 2). The soil nearer the trees showed greater deficits  
306 than that further away until September. Soil under the pruned trees showed smaller deficits  
307 compared to unpruned trees, particularly at 6 m distance. The maximum difference between  
308 treatments was approximately 40 and 20 mm for the canopy-reduced and canopy-thinned  
309 trees respectively (Figure 3).

310 Following recharging with water from winter rainfall, the soil under all trees  
311 remained near field capacity (i.e. zero deficit) until spring of year 2. The soil moisture deficits  
312 increased to their maximum in August (Figure 2), but differences between soil under the

313 pruned and unpruned trees were small except for the canopy-reduced trees at 6 m distance  
314 (Figure 2). Subsequently, the soil moisture deficit under all trees decreased, although the soil  
315 under unpruned trees contained less moisture and never achieved field capacity during  
316 winter. Lower winter rainfall (Figure 1) coincided with the soil under the unpruned trees not  
317 fully recharging below 60 cm depth (data not shown).

318 In year 3, differences in the soil moisture deficit (Figure 2) between the pruning  
319 treatments were small except for the canopy-reduced trees at 6 m distance, where the  
320 maximum difference from the unpruned trees was approximately 20 mm (Figure 3). A  
321 Friedman test applied to the means of the maximum difference from unpruned trees of soil  
322 moisture deficits for the 2 m and 6 m distance data across all years (Figure 2) was significant  
323 ( $p < 0.05$ ) for the canopy-reduced trees, whereas for the canopy-thinned trees it was not  
324 significant.

325 In experiment 2, the change in the soil moisture deficit due to pruning was first  
326 evident in June, when the soil under the severely canopy-reduced trees had a smaller  
327 moisture deficit than that under unpruned trees (Figure 2). As the deficit developed during  
328 the summer larger differences were apparent than in year 1 of experiment 1 (Figures 2, 3).  
329 By August the soil profile under severely canopy-reduced trees had a moisture deficit  
330 approximately one third less than that under the unpruned trees. In contrast to experiment  
331 1, the soil nearer the trees showed similar maximum differences from unpruned trees to  
332 that further away. These large differences in soil moisture deficit (approximately 50 mm)  
333 remained until autumn rainfall. Wilcoxon signed-rank tests applied to the means of the  
334 maximum difference of soil moisture deficits from unpruned trees for the 2 m and 6 m  
335 distance data (Figure 2) were significant ( $p < 0.001$  and  $p < 0.01$  respectively).

336 Therefore soil moisture content was consistently greater (i.e. a lower deficit was  
337 maintained) under canopy-reduced trees compared to unpruned trees. Following dry  
338 winters the soil under unpruned trees was not replenished to field capacity.

339

340 *Canopy manipulation - effects on regrowth and leaf nitrogen concentration*

341

342           The mean leaf size for canopy-reduced trees increased with time and was  
343 significantly different compared to unpruned trees in years 1 and 2 following pruning in  
344 experiment 1 (Table 1). After the severe canopy reduction in experiment 2, mean leaf size  
345 was substantially increased by canopy reduction compared to unpruned trees both for  
346 shaded and exposed leaves.

347           The canopy reduction and thinning treatments decreased canopy volumes by 72%  
348 and 6% respectively (data not shown). Unpruned trees had developed 30% of their canopy  
349 leaf area by May achieving full canopy around August. At 'full canopy', total leaf areas of the  
350 canopy-reduced and canopy-thinned trees were approximately one third and two thirds  
351 respectively of the unpruned trees (Table 2). LAD's of pruned trees showed no differences to  
352 unpruned trees. The drip line LAI indexes of canopy-reduced and canopy-thinned trees were  
353 similar to each other and the unpruned trees.

354           At 'full canopy' in the second year both pruning treatments had recovered a large  
355 proportion of their leaf areas. The CLA of the canopy-reduced and canopy-thinned trees  
356 were approximately 70 and 85% respectively of those of the unpruned trees (Table 2). LAD  
357 for the canopy-reduced trees was more than double that of the previous year. The mean leaf  
358 size on a branch sample from the canopy-reduced trees (274 cm<sup>2</sup>) was approximately  
359 double that found in the unpruned (138 cm<sup>2</sup>) and canopy thinned trees (106 cm<sup>2</sup>)  
360 respectively. This effect was significant ( $p < 0.05$ , SED 6 df, 30.6). The increase in LAD was due  
361 to the canopy-reduced trees growing their recovered CLA of larger leaves within a smaller  
362 volume than either of the other two treatments. The LAD's of the canopy-thinned and  
363 unpruned trees were similar.

364 At 'full canopy' in year 3, the canopy-reduced and canopy-thinned trees had fully  
365 recovered their CLA compared to the unpruned trees. The canopy-reduced trees continued  
366 to maintain denser canopies than either of the other treatments (Table 2). The average size  
367 of individual leaves was similar between all treatments indicating that the previous  
368 invigoration caused by the canopy reduction treatment had declined (Table 1).

369 In experiment 2, the severe canopy reduction (90% crown volume), greatly reduced  
370 subsequent CLA development (Table 2). The pruned trees had recovered only 25% of their  
371 CLA compared to the unpruned trees at 'full canopy'. The trees almost trebled their LAD in  
372 response to the pruning treatments. For both experiments canopy reduction increased leaf  
373 N concentration significantly directly after pruning (Table 1). The observed increase in leaf N  
374 concentration was greatest in experiment 2 for exposed leaves when compared to leaves  
375 from unpruned trees.

376

#### 377 *Canopy manipulation - effects on leaf water relations*

378

379 In mid-September, year 4, (i.e. when the soil was at its driest in experiment 2), only a small  
380 non-significant reduction in pre-dawn  $\psi$  of the severely canopy-reduced trees was measured  
381 (Table 1). Despite marked differences in soil maximal deficits (see Figure 1) the canopies of  
382 both canopy manipulation treatments restored leaf  $\psi$  overnight.

383 The leaf  $\psi$  at mid-day for the severely canopy-reduced trees were similar to those of  
384 the unpruned trees (Table 1). However,  $g_s$  of leaves sampled mid-day for the unpruned trees  
385 was significantly lower than for that of severely canopy-reduced trees (Table 1). Therefore,  
386 the latter trees did not restrict water use by stomatal closure. This is consistent with initial  
387 post-pruning effects found in other experiments on cherry trees (Dunn 2005; Hipps et al.  
388 unpublished data).

389

390 *Canopy manipulation - effects on stable isotope discrimination*

391

392 Bulk leaf  $\Delta^{13}\text{C}$  (carbon isotope discrimination) of trees was similar irrespective of treatment  
393 (Table 1). It was not until severe pruning was applied, in experiment 2, there was a shift in  $\Delta$   
394  $^{13}\text{C}$  of nearly 2‰ between exposed leaves of unpruned and severely canopy-reduced trees.  
395 Shaded leaves showed greater  $\Delta^{13}\text{C}$  than exposed leaves (Table 1). An interaction occurred  
396 between leaf canopy position and pruning treatment. Leaves from exposed or shaded  
397 positions, in canopy-reduced trees, showed similar  $\Delta^{13}\text{C}$ , whereas a large difference occurred  
398 between unpruned trees in these positions.

399 The results for leaf  $\Delta^{18}\text{O}$  were consistent, as more evaporative enrichment occurred  
400 in all years in experiment 1 in the canopy-thinned and unpruned trees than for the canopy-  
401 reduced trees. The organic signal  $^{18}\text{O}$  data were also consistent with evaporative enrichment  
402 in canopy-thinned trees when sampled in year 1 (data not presented). A similar result for  
403 leaf  $\Delta^{18}\text{O}$  was found for experiment 2. However, the effect was significantly larger for  
404 exposed than for shaded positions. An attempt was made to use  $^{18}\text{O}$  to determine the  
405 sources of water in the tree. These data did not show any discrimination between rainwater  
406 samples and tree water, indicating that rainwater (root extracted from the upper soil  
407 horizon) was the main source of transpiration water (data not shown).

408

409 **Discussion**

410

411 *Canopy manipulation effects on soil moisture*

412

413 Canopy architecture, its manipulation, tree size and planting density all impact on  
414 transpiration (Tyree and Ewers 1991). Water use in isolated trees, particularly in urban  
415 environments, may differ markedly from that in continuous canopies (Kjelgren and Clark



416 1992, Kjelgren and Montague 1998). However, canopy manipulation is carried out to reduce  
417 soil drying and assumes there is a functional relationship between CLA and tree water use.  
418 This assumption seems reasonable, but it would be highly dependent on factors such as LAD,  
419  $g_s$  and canopy conductance. Moderate pruning may reduce LAD to a point where light  
420 interception is still at an optimal LAI. Pruned tree canopies may be more effectively coupled  
421 to the atmosphere and this increases transpiration rather than decreases it (Jarvis and  
422 McNaughton 1986; Wullschlegel et al. 2000). Radical reduction of CLA (>85%) may be  
423 required to reduce tree water use and improve soil water recharge (Jackson et al. 2000). In  
424 our experiments reducing CLA correlates with conservation of soil moisture below the tree  
425 and is assumed to be due to a decline in tree water use.

426         Research on tree root architecture in urban settings is very limited with most studies  
427 on forest or orchard settings or on immature trees. Using a meta-analysis of 19 studies on a  
428 range of coniferous and deciduous species, Day et al. (2010), found that trunk diameter  
429 accounted for 89% of the variation in root spread. Similar analysis using tree height as the  
430 determinant only accounted for 36% of the variation in root spread. Day et al. (2010)  
431 estimated that the radius of an unrestricted root system increases at a rate of 38:1  
432 compared to trunk diameter, however, for the included species this ratio considerably  
433 declines as the trees mature. The relationship reached an asymptote as the trunk diameter  
434 approaches 25 - 30 cm, and root system radius remains at about 5 m even as the trunk  
435 diameter increases to 70 cm. However, the Day et al. (2010) model did not include London  
436 plane trees and the data of damage to building caused by the proximity of this species  
437 recorded by Cutler and Richardson (1997) implied that their root systems radii can regularly  
438 exceed 5 m. Seventy per cent canopy volume reduction showed greater soil moisture  
439 conservation at 6 m from the tree and this suggests that the tree roots in this experiment  
440 spread at least this far and were within the distance range that caused a high frequency of  
441 damage to buildings on swelling clay soils according to Cutler and Richardson (1997).. Trees

442 of the size used here have a large proportion of root capable of water uptake, at the  
443 periphery of their root system (e.g. Atkinson 1980). Severe canopy reduction (90% canopy  
444 volume) increased soil moisture conservation closer to the trunk and confirms that root  
445 system responses are linked to the severity of pruning. Longer-term changes in soil moisture,  
446 with respect to reducing tree water use, also involve 'recharging' which is dependent on  
447 winter rainfall. Our data show when winter recharging (during November and December)  
448 was below average, canopy manipulations effects on soil moisture conservation were  
449 prolonged. Treatment variation in the rate with which CLAs developed, particularly when it  
450 involved the production of new meristematic apices, also influenced the timing of soil  
451 moisture conservation.

452

#### 453 *Canopy manipulation effects on vegetative growth*

454

455 Canopy pruning and defoliation can change carbon allocation patterns (Mediene et  
456 al. 2002, Cerasoli et al. 2004). Reducing leaf area can result in vigorous canopy regrowth and  
457 re-establishment of the initial root:shoot ratio. Observations of roots, after canopy  
458 reduction, show growth to have declined along with reducing sugars, while root death  
459 increases (Head 1969, Atkinson 1980, Eissenstat and Duncan 1992). This response can be  
460 rapid, for example, with *Acer saccharium*, the decline in shoot photosynthesis was linked  
461 with a measurable reduction of root growth within 24 h (Kozlowski 1971b). Roots, and total  
462 leaf area, can recover from canopy reductions within a few months, but starch reserves can  
463 be depressed for several months after treatment (Head 1969, Eissenstat and Duncan 1992).  
464 The duration of canopy treatment effects, and species differences in root longevity,  
465 contribute to system responses observed, along with the time the canopy manipulation is  
466 applied (Head 1969, Kozlowski 1971b, Atkinson 1980, Cerasoli et al. 2004, Mediene et al.  
467 2002, Tschaplinski and Blake, 1995). Root system capacity to supply a given CLA might be

468 expected to be optimized for a given tree species and its above and belowground  
469 environment (Weiner 2004). It is however challenging to find experimental support for this  
470 notion. CLA reduction produces a supra-optimal root system with a greater capacity to  
471 supply, initially, a smaller leaf canopy. The initial maintenance of the root systems requires  
472 factors derived from the activity of new shoot growth apices and in their absence root  
473 growth will decline and in turn shoot growth also will decline (Morris 1996, Head 1969).

474 Our canopy-reduced trees showed rapid shoot regrowth and a large recovery of CLA  
475 within three years after treatment. There was, however, still a significant reduction in CLA  
476 apparent for canopy-reduced trees present at the end of year two. Regrowth generally  
477 occurred via the initiation of new shoot apices ('epicormic' growth) and the release of lateral  
478 buds on shoots without apical buds (Kozlowski 1971b). These 'lateral bud' leaves had similar  
479 mean leaf areas and shoots with a determinate leaf number (Kozlowski 1971a). Epicormic  
480 regrowth, for canopy-reduced trees, produced leaves with larger mean areas, higher N  
481 concentrations, and shoots of indeterminate growth, which facilitated the rapid recovery of  
482 CLA and an increase in LAD. Removal of entire branches (canopy thinning) leaving branches  
483 with apical buds intact had no stimulatory effect on the rest of the canopy ((Kozlowski  
484 1971ab). Thinned canopies switched to a more indeterminate growth form after terminal  
485 buds set and then broke to produce heterophyllous shoots (Kozlowski 1971a). The key  
486 observation in this study was that differences in leaf size and LAD do not translate into  
487 differences in soil moisture content and likely tree water use, since the dense regrowth  
488 associated with canopy reduction uncouples the canopy from the atmosphere, as we explore  
489 by comparing leaf-level differences in  $g_s$  and isotope discrimination below.

490

491 *Canopy manipulation effects on stable isotopes*

492

493 The lack of any differences in bulk leaf  $\Delta^{13}\text{C}$  with experiment 1, over time irrespective of  
494 treatment, implies that water use inferred from stomatal conductance ( $g_s$ ) was not  
495 influenced over the seasonal life of the leaf, or longer-term by canopy regrowth or  
496 subsequent architectural changes (Seibt et al. 2008). However, there were different effects  
497 when measurements were made in the dense regrowth after severe canopy reduction,  
498 consistent with the offset seen in sun and shade leaves in the unpruned treatment (Table 1).  
499 For exposed leaves, which are from comparable positions to leaves sampled in experiment 1,  
500  $\Delta^{13}\text{C}$  values in the severe canopy reduction treatment were higher, consistent with the  
501 significant increase in  $g_s$  in these leaves (Table 1). This increase in  $\Delta^{13}\text{C}$  and  $g_s$  was  
502 accompanied by a more than doubling of mean leaf size, and LAD despite the severe canopy  
503 reduction. We suggest that the dense canopy boundary layer leads to uncoupling of leaf  
504 water use and  $g_s$  from atmospheric conditions. This, is similar to the response of  $\Delta^{13}\text{C}$  seen in  
505 deep shade (see also Table 1, unpruned trees), whereby under limiting light,  $\Delta^{13}\text{C}$  is  
506 uncoupled from water use (Seibt et al. 2008). Therefore, changes in leaf canopy structure  
507 and canopy LAD have had a greater effect on transpiration and ETp (reflected in soil  
508 moisture profiles) rather than  $g_s$  alone, due to differences in atmospheric coupling (Jarvis  
509 and McNaughton 1986, Wullschleger et al. 1998, 2000). Due to the size of the trees used in  
510 these experiments we were unable to measure coupling extensively throughout the canopy  
511 however in other experiments with smaller *Prunus* which responded to similar pruning  
512 treatments in a very similar way we did (Dunn 2005). These experiments used an array of  
513 evaporation sensors to simultaneously determine leaf boundary layer conductance variation  
514 within the canopy. Results showed that canopy reduction reduced boundary layer  
515 conductance while canopy thinning increased it (Dunn et al. unpublished). Bulk leaf water  
516  $\delta^{18}\text{O}$  discrimination provides support for the notion that changes in leaf coupling are a  
517 consequence of canopy reduction (Farquhar and Cernusak 2005, Cernusak and Kahman,  
518 2013). In experiment 1 and 2, the reduction in  $\delta^{18}\text{O}$  leaf water enrichment in each year after

519 pruning is consistent with a reduction in evaporative demand and leaf water loss in the  
520 dense regrowth with high LAD and CLA recovery.

521 Direct measurement of soil moisture under severe canopy-reduced trees showed  
522 considerably greater soil moisture conservation, particularly at 6 m from the tree. The  
523 consistency of short-term soil moisture conservation seen with both experiments indicates  
524 canopy-reduced trees used less water, and re-emphasises the need to interpret  $\Delta^{13}\text{C}$  as a  
525 measure of instantaneous water use efficiency with care within forest and crop canopies  
526 when influenced by low light and/or canopy uncoupling (Seibt et al. 2008). It would also  
527 explain why canopy thinning, with increased boundary layer conductance, and atmospheric  
528 coupling, showed promoted water use. With respect to our original hypothesis we can  
529 conclude that in general terms the advised level of tree canopy reduction applied in the UK  
530 does in fact reduce tree water use, whereas canopy-thinning has no effect. However, a large  
531 reduction in tree water use is short-lived and suggests that frequent reapplication of canopy  
532 treatments will be required to ensure maximum soil moisture conservation. The regularity of  
533 pruning will likely be dependent on a species ability to regrow as well as its environment.  
534 Stimulation of canopy regrowth, with London plane, produced changes in shoot architecture  
535 and leaf morphology which induced a rapid reestablishment of leaf canopy area and this  
536 reduced the duration of soil moisture conservation. The use of stable isotopes, support the  
537 idea that tree canopy modifications influence functional aspects of tree water use and that  
538 recovery in canopy leaf area was countered by reduced coupling to the surrounding  
539 atmosphere. Rainfall patterns, particularly, during winter soil recharging were also important  
540 in determining the duration of treatment effects. To achieve greater and longer-term soil  
541 moisture conservation however requires severe canopy reductions.

542

543 **Conclusions**

544

545 These experiments show that when winter soil water recharging was below average, canopy  
546 manipulation effects on soil moisture conservation were prolonged. While, treatment  
547 differences in canopy leaf development, through the production of meristematic apices,  
548 delayed water use promoting soil moisture conservation. Canopy-reduced trees used less  
549 water and conserved more soil moisture initially, but this advantage was lost as they rapidly  
550 regrew and recovered their leaf area within three years of pruning. Treatment differences in  
551 leaf size and leaf area density did not translate into differences in soil moisture content as  
552 dense canopy regrowth was uncoupled the external canopy atmosphere as shown by a  
553 reduction in  $\Delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  leaf water enrichment. Large reductions in tree water use were  
554 short-lived and imply that frequent reapplication of canopy reduction treatments is required  
555 to maximum soil moisture conservation.

556

557

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559

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567

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## Tables

**Table 1** Changes due to canopy manipulation treatments, applied to London plane (*Platanus x acerifolia*) trees, in individual leaf size, leaf nitrogen concentration,  $\Delta^{13}\text{C}$  bulk leaf and  $\Delta^{18}\text{O}_{\text{VSMOW}}$  leaf water concentrations for leaves sampled in late August and early September for experiments 1 and 2 respectively. Pre-dawn and mid-day measurements of leaf water relations in September for experiment 2. (SR = severely reduced)

Experiment 1	Year	Unpruned	Reduced	Thinned	SED <sup>a</sup>	$p^b$
Leaf size (cm <sup>2</sup> )	1	207	288	254	23.8	<0.01
	2	220	448	227	51.3	<0.01
	3	364	331	276	44.4	ns
Nitrogen (g m <sup>-2</sup> )	1	1.5	2.9	1.8	0.25	<0.001
	2	2.0	2.4	1.7	0.54	ns
	3	2.0	1.7	1.7	0.11	ns
$\Delta^{13}\text{C}$ bulk leaf (‰)	1	19.0	18.9	18.3	0.30	ns
	2	18.8	19.1	19.1	0.70	ns
	3	19.4	19.6	19.7	0.28	ns
$\Delta^{18}\text{O}_{\text{VSMOW}}$ (‰) leaf water	1	10.3	7.0	8.7	1.98	ns
	2	6.4	3.7	5.0	1.25	<0.05
	3	15.8	13.0	14.5	1.05	ns

<sup>a</sup>Year 1 & 2, SED are for comparison between unpruned and reduced treatments only

<sup>b</sup>t test used to test significance in year 1 & 2, thinned treatment ns for all measurements, ANOVA used in year 3

679

Experiment 2		(Year 4)						
Canopy position	Shaded		Exposed		SED (4 df)	$p^c$ (Red)	$p^c$ (Pos)	$p^c$ (Red x Pos)
	Un-pruned	SR	Un-pruned	SR				
Leaf size (cm <sup>2</sup> )	245	502	209	667	85.9	<0.05	ns	<0.05
Nitrogen (g m <sup>-2</sup> )	0.94	1.7	1.7	2.2	0.20	0.052	<0.01	ns
$\Delta^{13}\text{C}$ bulk leaf (‰)	22.3	21.3	18.5	20.3	0.39	ns	<0.001	<0.01
$\Delta^{18}\text{O}_{\text{VSMOW}}$ (‰) leaf water	10.1	8.9	15.1	9.3	0.50	<0.05	<0.001	<0.001
Diel time course			Un-pruned	SR	SED (2 df)	$p^c$ (Red)		
Pre-dawn $\psi$ (MPa)			-0.193	-0.160	0.019	ns		
Mid-day $\psi$ (MPa)			-1.359	-1.215	0.076	ns		
$g_s$ (mmol m <sup>-2</sup> s <sup>-1</sup> )			79	282	32	<0.05		

<sup>c</sup>probabilities are *P Red* for the severe canopy reduction (SR) treatment and *P Pos* for canopy leaf position, only exposed leaves measured for  $\psi$  and  $g_s$ .

680

681 **Table 2** The effect of different canopy manipulation treatments applied to London plane  
 682 (*Platanus x acerifolia*) trees on total canopy leaf area (CLA), leaf area density (LAD) and  
 683 drip line leaf area index (LAI) measured with hemispherical images and HemiView image  
 684 analysis  
 685

Year	Month	Unpruned	Reduced	Thinned	SED (6 df)	<i>p</i>
<b>Canopy leaf area (m<sup>2</sup>)</b>						
1	May <sup>a</sup>	178	26	124	41.5	ns
	Jun <sup>a</sup>	351	106	193	117.2	ns
	Jul <sup>a</sup>	593	193	476	92.6	ns
	Aug	598	190	437	55.8	<0.001
2	July	750	426	699	62.6	<0.01
	Aug	756	522	660	49.9	<0.001
3	Sep	547	466	561	66.1	ns
Experiment 2		Unpruned	Severely reduced	Thinned	(8 df)	
4	Aug	630	141		32.8	<0.001
<b>Leaf area density (m<sup>2</sup> leaf m<sup>-3</sup> crown)</b>						
<b>Experiment 1</b>						
1	May <sup>a</sup>	0.098	0.063	0.065	0.0150	ns
	Jun <sup>a</sup>	0.185	0.253	0.188	0.0435	ns
	Jul <sup>a</sup>	0.333	0.453	0.255	0.0890	ns
	Aug	0.329	0.378	0.249	0.0491	ns
2	July	0.414	0.854	0.404	0.0745	<0.01
	Aug	0.425	1.044	0.388	0.0690	<0.001
3	Sep	0.406	0.661	0.443	0.0633	<0.05
Experiment 2		Unpruned	Severely reduced	Thinned	(8 df)	
4	Aug	0.526	1.048		0.1322	<0.01
<b>Drip line leaf area index (m<sup>2</sup> leaf m<sup>-2</sup> ground)</b>						
<b>Experiment 1</b>						
1	May <sup>a</sup>	1.05	0.43	0.72	0.167	ns
	Jun <sup>a</sup>	2.03	1.78	2.08	0.518	ns
	Jul <sup>a</sup>	3.54	3.17	2.80	0.782	ns
	Aug	3.59	2.68	2.71	0.413	ns
2	July	4.51	6.05	4.39	0.502	<0.05
	Aug	4.59	7.40	4.19	0.361	<0.001
3	Sep	4.44	5.98	4.80	0.616	ns
Experiment 2		Unpruned	Severely reduced		(8 df)	
4	Aug	6.00	5.54		0.864	ns

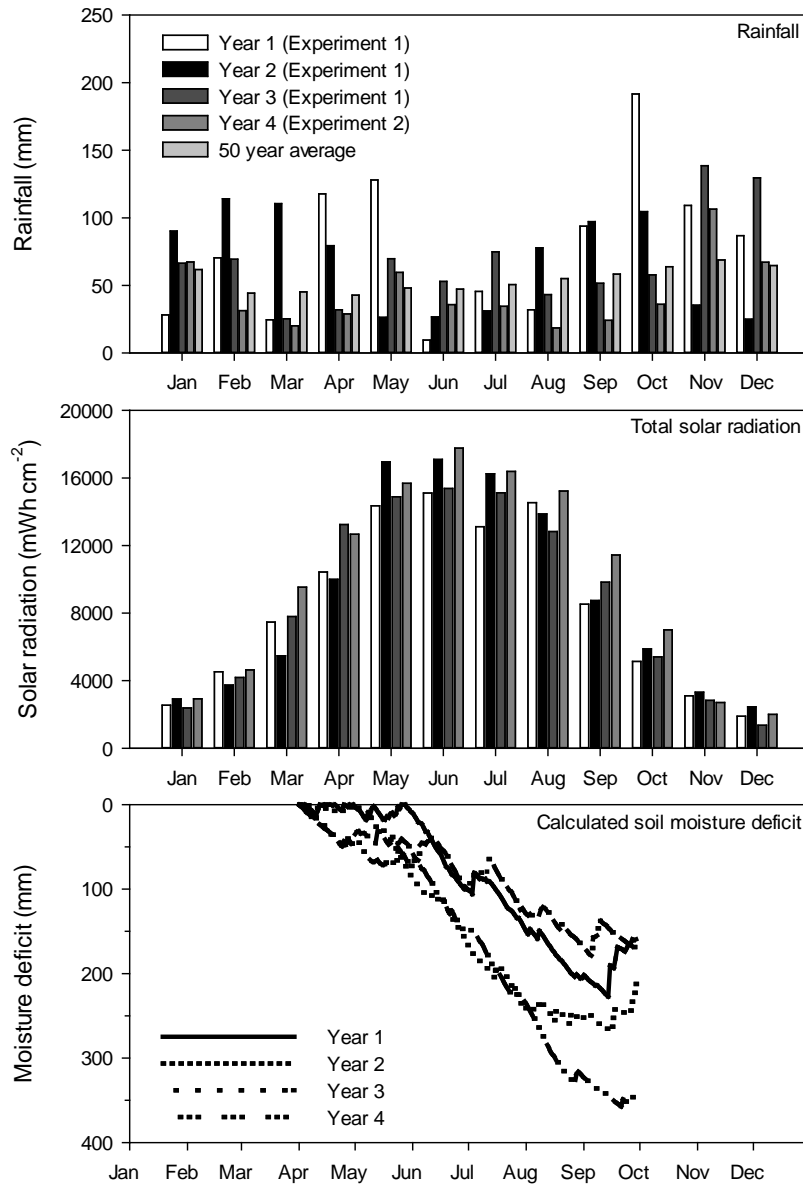
<sup>a</sup>df = 2

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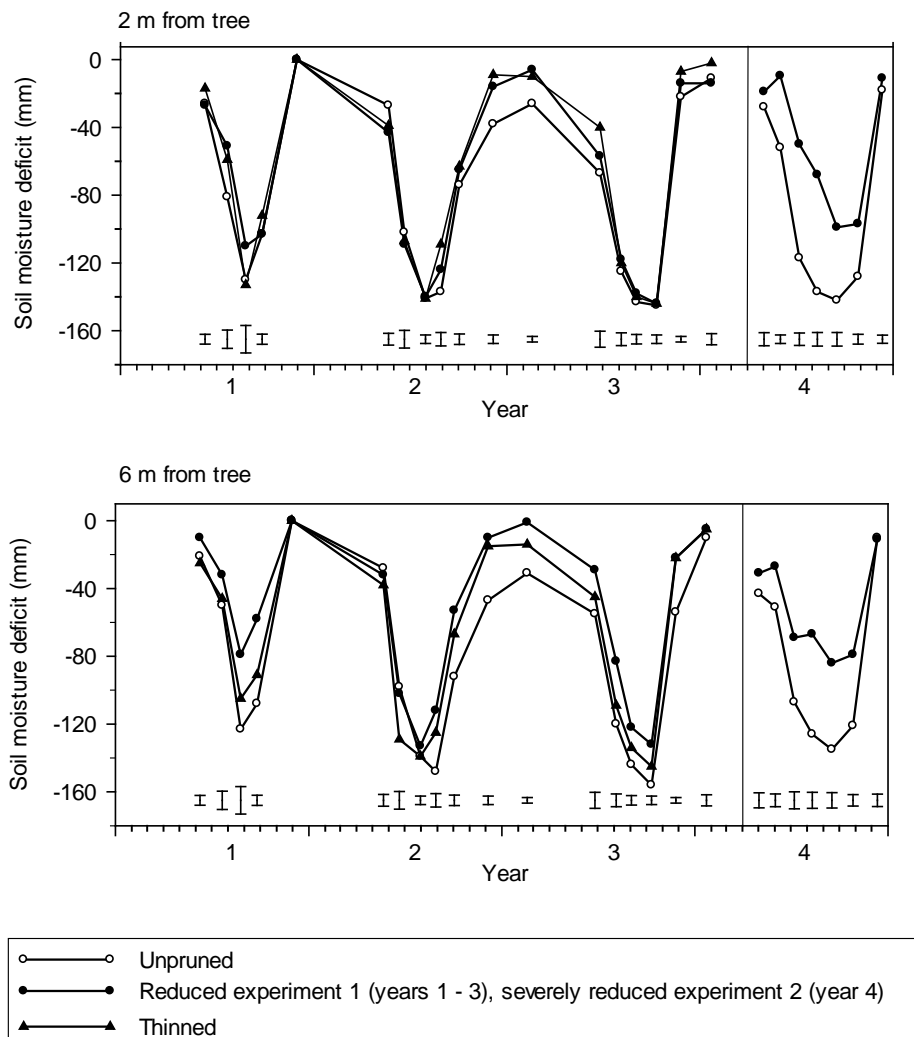
## Figures

**Fig 1** Monthly weather conditions including rainfall and the 50 year average, total solar radiation and soil moisture deficits at East Malling Research, over the four years of the experiments



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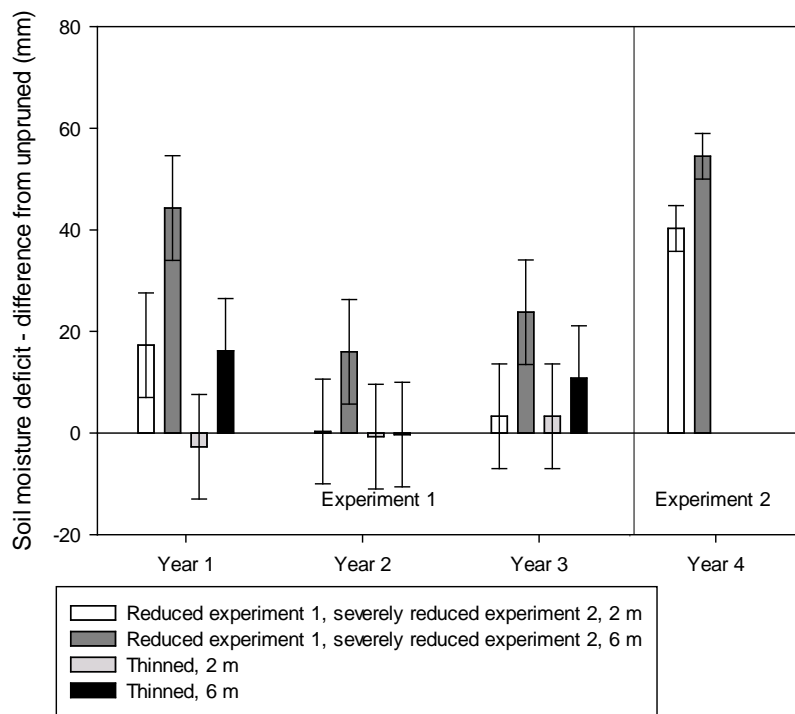
705 **Fig 2** The effect of canopy manipulation treatments for London plane (*Platanus x acerifolia*)  
 706 trees on the soil moisture deficits (0 – 100 cm), measured with a neutron probe, at 2 and 6  
 707 m from the trunk. (Vertical lines are SED's for each date separately)



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**Fig 3** The effect of canopy manipulation treatments for London plane (*Platanus x acerifolia*) trees on the difference in the maximum soil moisture deficit achieved when compared with

725 unpruned trees for the canopy-reduced and canopy-thinned trees in experiment 1 and  
 726 severely canopy-reduced trees in experiment 2. (Vertical lines are standard errors)  
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