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Original Citation:

Effects of different pruning methods on an urban tree species: a four-year-experiment scaling down from the whole tree to the chloroplasts / Fini, A.; Frangi, P.; Faoro, M.; Piatti, R.; Amoroso, G.; Ferrini, F.. - In: URBAN FORESTRY & URBAN GREENING. - ISSN 1618-8667. - STAMPA. - 14:(2015), pp. 664-674. [10.1016/j.ufug.2015.06.011]

Availability:

This version is available at: 2158/1003874 since: 2015-10-08T12:05:21Z

Published version:

DOI: 10.1016/j.ufug.2015.06.011

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Accepted Manuscript

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PII: S1618-8667(15)00093-X
DOI: <http://dx.doi.org/doi:10.1016/j.ufug.2015.06.011>
Reference: UFUG 25563

To appear in:

Received date: 18-9-2014
Revised date: 16-4-2015
Accepted date: 25-6-2015

Please cite this article as: Fini, A., Frangi, P., Faoro, M., Piatti, R., Amoroso, G., Ferrini, F., Effects of different pruning methods on an urban tree species: a four-year-experiment scaling down from the whole tree to the chloroplasts, *Urban Forestry and Urban Greening* (2015), <http://dx.doi.org/10.1016/j.ufug.2015.06.011>

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1 **Effects of different pruning methods on an urban tree species: a four-year-experiment scaling down**
2 **from the whole tree to the chloroplasts**

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15

16 **Abstract**

17 The aim of this work was to evaluate the effects of repeated pruning interventions using different pruning
18 methods on growth and physiology of *Acer pseudoplatanus* L. Trees were pruned in 2008 and 2010
19 according to widely used pruning techniques for urban trees, such as reduction cut, removal cut and heading
20 (topping) cut. Crown dieback, growth of the plant and of the pruned branches, leaf morphological traits and
21 leaf gas exchange were assessed during the two growing season after each pruning cycle. Topping cut (i.e.
22 the pruning treatment which suppressed the primary axis without providing a substitute) induced changes on
23 tree growth pattern (i.e. by increasing the release of adventitious watersprouts and root suckers and
24 decreasing stem diameter growth), which were not observed in the other pruning treatments. At the leaf level
25 only topping cut increased leaf area at the expense of leaf mass per area, which may contribute to explain the
26 higher occurrence of dieback on topped branches than in control and in the other pruning treatments. Also,
27 leaves on topped branches displayed higher chlorophyll content and higher activity of Calvin cycle enzymes,
28 which did not translate in higher CO₂ assimilation. We show here that pruning method, not only its severity

29 (i.e. the amount of leaf area removed), modulates the morpho-physiological response of trees to pruning and
30 that maintenance of apical control and apical dominance are key issues to preserve a structurally sound tree
31 structure, as well as the long-term efficiency of the photosynthetic apparatus.

32

33 **Key words:** *Acer pseudoplatanus*, apical control, leaf gas exchange, reduction cut, removal cut, topping cut

34

35 **Introduction**

36 Trees growing in the urban environment require periodic pruning to provide clearance and improve view (i.e.
37 trees along roadsides), to reduce conflicts with buildings and infrastructures, to thin dense canopies and
38 decrease wind resistance, and to reduce risk of failure by removing structural defects (Dureya et al., 1996;
39 Smiley and Kane, 2006).

40 Three types of pruning cuts are commonly used to prune urban trees (American National Standard Institute,
41 2008; Gilman, 2012): removal cut, reduction cut and heading (topping) cut. Removal cut removes the whole
42 target branch at its attachment to the trunk or parent branch, thus eliminating the entire lateral growing axis.
43 Reduction cut shortens the primary axis by removing the distal end to a smaller lateral branch, which should
44 be at least one-third to one-half of the diameter of the removed portion and should assume the apical role for
45 the remaining branch (Harris et al., 2004; Grabosky and Gilman, 2007). Finally, topping cut shortens the
46 primary axis by cutting the distal portion of the branch in the internode or in between consecutive lateral
47 branches. In this case, no properly-sized lateral is retained to assume the role of apical growing axis for the
48 remaining branch (Harris et al., 2004). These pruning methods differ in the way the target branch and its
49 apical portion are managed. It is long known that apical buds (of the tree main stem and of individual
50 branches) control key physiological processes determining tree structure and growing pattern (Cline 1994,
51 1996). These include apical dominance (i.e. the inhibition of lateral bud sprouting by the apex in an
52 individual branch) and apical control (i.e. the influence of apical growing axis on elongation and orientation
53 of lateral shoots within an individual branch) (Martin, 1987; Cline, 1997). Much of research on pruning of
54 urban trees, however, has focused on pruning severity and timing (Mierowska et al., 2002; Gilman and

55 Grabosky, 2009; Fini et al., 2013), on tree response to wounding (Solomon and Blum, 1977; Neely, 1979;
56 Schwarze, 2008), on compartmentalization of wood decay fungi (Shigo and Marx, 1977; Schwarze, 2001;
57 O'Hara, 2007; Schwarze et al., 2007) or on tree response in the wind (Gilman et al., 2008a, 2008b; Pavlis et
58 al., 2008), whereas very little research has focused on the effects of pruning method on the long-term
59 structure and physiology of urban trees (Clark and Matheny, 2010). Because of the lack of knowledge about
60 the long-term physiological effects of pruning, it is not possible to set national and international standards
61 and best practices aimed at improving tree health and longevity and, in several countries, pruning
62 prescriptions are mostly based on operational needs and short-term cost criteria (Campanella et al., 2009;
63 Maurin and DesRochers, 2013).

64 Most of the research investigating physiological and growth response to pruning has been conducted on fruit
65 or timber trees (Lebon et al., 2004; Spann et al., 2008; Fumey et al., 2011; Maurin and DesRochers, 2013),
66 but these findings may not be directly transferred to urban trees because pruning aims are completely
67 different [i.e. improving fruit yield or quality and producing clearwood for fruit and timber production,
68 respectively, while urban arboriculture is primary targeted to obtain large, healthy, long-lived trees with a
69 sound structure, capable of providing large benefits to the community, see Nowak et al. (2002)]. Research on
70 fruit and timber plantations showed that pruning stimulates emission of new sprouts from latent and
71 adventitious buds on the pruned branch, depresses plant height and stem diameter growth, and depletes non-
72 structural carbohydrates pool (Davidson and Remhprey, 1994; Spann et al., 2008), but the implications of
73 these morphological changes to long-term structural soundness were beyond the aims of these works.
74 Photosynthesis is also affected by pruning, often showing temporary increases (the so called "compensatory
75 photosynthesis"), the extent of this increase being usually related to the amount of leaf area removed
76 (Pinkard and Beadle, 1998; Medhurst et al., 2006). Whether the increase in photosynthesis is related to
77 increased leaf nutrients and chlorophyll, to higher carboxylation efficiency and ribulose regeneration, to
78 higher stomatal conductance, to the depletion of nonstructural carbohydrates pool or to altered source:sink
79 ratio is still a matter of debate (Pinkard et al., 1998; Lavigne et al., 2001; Li et al., 2002; Turnbull et al.,
80 2007).

81 The aim of this work was to evaluate the long-term effects of different pruning methods on the structure of
82 the whole tree and of the pruned branches, as well as the effects on selected leaf traits and leaf gas exchange.
83 We hypothesized that pruning method, not only its severity (i.e. the amount of leaf area removed, see
84 Pinkard and Beadle, 1998; Medhurst et al., 2006), can modulate tree response, and that greater reaction to
85 pruning by the tree may occur in treatments which mostly suppress apical control and dominance. In detail,
86 we tested the following hypotheses: 1) topping cut may completely impair apical control and dominance,
87 thereby promoting release of lateral sprouts from latent or adventitious buds and increasing the occurrence of
88 codominant branching on the pruned branches; 2) reduction cut may, instead, preserve apical dominance and
89 control, thereby resulting in much lower disturbance to tree structure; 3) the removal of the whole branch to
90 its attachment to the trunk will provide minimal disturbance to tree morphological and physiological
91 processes, because regrowth may be avoided by apical dominance exerted by the trunk; 4) all pruning
92 treatments will induce similar increases in leaf biochemical parameters and photosynthetic rate, but
93 competition among codominant sprouts will result, in the long-term, in greater decline of photosynthesis in
94 topped trees; 5) pruning effects on trees will increase as pruning is repeated over time.

95

96 **Materials and Methods**

97 *Plant material and environmental conditions*

98 In spring 2005, 28 uniform 3.2-3.8 cm diameter (10-12 cm circumference) sycamore maples (*Acer*
99 *pseudoplatanus* L.) were planted in an experimental plot at the Fondazione Minoprio (Vertemate con
100 Minoprio, Como, Italy; 45°44' N, 9°04' E), in a loamy sand, well drained soil. Mean annual rainfall in the
101 experimental site, calculated over the last 20 years, is 1086 mm and average temperature 12.3 °C. Daily
102 temperature and rainfall were recorded using a weather station (Vantage Pro 2, Davis, San Francisco, CA,
103 U.S.) throughout the experimental period (monthly average temperature and total rainfall are reported in Fig.
104 1). Mean yearly rainfall recorded during the experiment was greatly above the 20-year average except for
105 2011 (867 mm total rainfall), whereas mean yearly temperature was close to the 20-year average throughout
106 the experiment.

107 *Pruning treatments and experimental set up*

108 After planting, trees were allowed to establish and grow undisturbed for 3 years. In February 2008 (1st
109 pruning cycle), plants were pruned using bypass hand pruners, according to the following treatments
110 (illustrated in Fig. 2): 1) Topping cut: pruning cuts were made in the middle of the internode of first-order
111 lateral branches (over 3-year old); 2) Removal cut: first-order lateral branches (over 3-year old) were cut at
112 their union with the stem, using care not to damage branch collar (Shigo, 1990); 3) Reduction cut: first-order
113 lateral branches (over 3-year old) were cut back to a lateral with sufficient size to become a new leader.
114 Therefore, all new leaders chosen had aspect ratio (calculated as ratio between the diameter of lateral chosen
115 as new leader and that of the parent branch to be removed, both measured above the branch union) greater
116 than 0.33 (Gilman, 2012); 4) Control: plants were left unpruned. In February 2010 (2nd pruning cycle), trees
117 were pruned again according to the same treatments as in 2008. All cuts were made at nodes or internodes
118 which were over 2-year old. Following the recommendations by ANSI A300 (American National Standard
119 Institute, 2008), regardless of pruning method, pruning was carried out in order to reduce leaf area by 30%,
120 which corresponds to a mild defoliation (Willard and McKell, 1978; Simard et al., 2012). Because trees were
121 pruned during the dormant season, branch cross sectional area was used to estimate the amount of leaf area
122 removed (Grabosky et al., 2007; Gilman and Grabosky, 2009). Also, while pruning, pruned material was
123 weighed in order to confirm the removal of a similar amount of woody biomass in all pruning treatments.
124 The weight of the pruned material was 1438 ± 355 g and 2088 ± 492 g, in the first and in the second pruning
125 cycles, respectively, and was not affected by pruning method ($P = 0.333$ and 0.393 in the first and in the
126 second pruning cycles, respectively). To remove the same amount of wood, removal cut required about 50%
127 less pruning cuts than topping cut and 35% less than reduction cut. In both pruning cycles six pruning cuts
128 per plant (42 per treatment) were marked with paint to be recognizable for subsequent measurements. In
129 control trees, six imaginary cuts were drawn on first-order lateral branches, similar in size and age as those
130 used in pruned treatments. Imaginary cuts were drawn next to a lateral having aspect ratio greater than 0.33
131 compared to the parent branch. In treatments where the apical bud of the branch was retained (i.e. control) or
132 substituted (i.e. reduction), the shoot bearing that apical bud was considered the leader of the branch. In
133 treatments which suppressed the apical bud (i.e. topping and removal cuts), the longest (after the first

134 growing season) of the newly developed sprouts was considered as the new branch leader, while the
135 remaining were considered as laterals (Fig. 2).

136

137 *Biometric measurements, wound closure and breaking stress*

138 In both pruning cycles, all biometric parameters and wound closure were measured at the time of pruning,
139 and 12 and 24 months after pruning. Wound closure was estimated using the woundwood coefficient
140 (Scwharze, 2008), $WC = 100 - [(\pi/4) * b_{t1} * h_{t1} * 100] / [(\pi/2) * (r_{t0})^2]$, where: b_{t1} and h_{t1} are the width and the
141 height of the wound at the time of measurement, and r_{t0} is the initial radius of the pruning wound.

142 Stem diameter was measured at 1.3 m and stem Relative Growth Rate (RGR_{stem}) was calculated as $[\ln(\varnothing_{t1}) -$
143 $\ln(\varnothing_{t0})] * (t_1 - t_0)^{-1}$ where: \varnothing is stem diameter at times 0 and 1, and $t_1 - t_0$ is time (in days) between
144 measurements (Newbery et al., 2011). The number of root suckers developed was counted in each replicate
145 tree. Then, the relative frequencies were calculated, in each treatment, as the ratio between trees releasing a
146 certain number of root suckers (i.e. 0, 1 to 4, 5 to 7, more than 7) over total number of trees of that treatment.
147 Twelve and twenty-four months after each pruning cycle, slenderness of the whole branch ($L:D_{wb}$) was
148 calculated as the ratio between the length and base diameter of pruned branches. Branch length was
149 measured from its attachment to the trunk to the apical bud, while the base diameter was measured at the
150 union with the trunk. Crown dieback was assessed visually 6 and 17 months after each pruning cycle. Crown
151 dieback frequency was calculated, in each replicate tree, as the ratio between pruned branches showing
152 dieback symptoms (i.e. extensive leaf necrosis, absence of growth, bud death) over the total number of
153 marked branches of that treatment. A pruned branch was counted for dieback if showing any of the above
154 mentioned signs on any part of the branch, including sprouts released after pruning.

155 The number, base diameter and length of watersprouts developed or released within 20 cm (as suggested by
156 Grabosky and Gilman, 2007) from the pruning cut or at the callus dieback line were measured 12 and 24
157 months after pruning. Within each marked pruned branch, the slenderness of the leader ($L:D_{leader}$) was
158 calculated as the ratio between the leader length and base diameter. Similarly, the slenderness of the lateral
159 shoots/sprouts ($L:D_{lateral}$) was calculated as the average of the slenderness of all individual sprouts

160 (excluding, in topping and removal, the sprout designated as new leader) released from the pruning cut.
161 Length of the leader and lateral shoots/sprouts was measured from their attachment on the higher order
162 branch (in most cases, from the pruning cut) to the apical bud, while diameter was measured just above the
163 attachment. The aspect ratio between the lateral and the leader was calculated as the ratio between the base
164 diameter of each lateral shoot and base diameter of the leader.

165 The stress (σ) required to cause the failure of the attachment between the leader of the branch (or the selected
166 lateral, in control trees) and the parent branch was measured using the methods proposed by Kane et al.
167 (2008). Twenty-four months after each pruning cycle, 14 branch unions per treatment (56 in total in each
168 cycle) were attached to a dynamometer (HCB 200, Kern and Sohn GmbH, Balingen, Germany), loaded at a
169 rate of 5 cm per minute until breakage of the attachment. Breaking stress (σ) was then calculated as: $32 * P * L * \sin\alpha / (\pi * d^3)$ where: P (kN) is the maximum load; L (m) is the distance between the point of application
170 of the load and the attachment which was kept fixed (about 5 cm); d (m) is the inside-bark branch diameter;
171 α (rad) is the angle between the longitudinal axis of the branch and the applied load.
172

173

174 *Leaf gas exchange and integrated leaf anatomical traits*

175 Five and seventeen months after each pruning cycle, after leaves had reached their final size, 10 fully
176 expanded leaves per tree (70 leaves per treatment) were harvested from the leader shoot/sprout of pruned
177 branches and immediately scanned using an A-3 scanner. An image analysis software (Image Tool v1.3,
178 University of Texas, San Antonio, TX, U.S.) was used to measure average leaf area. Leaves were then oven-
179 dried at 70°C until constant weight to determine dry mass. Then, leaf mass per area (LMA) was calculated as
180 the ratio between leaf dry mass and leaf area. Leaf greenness index, a parameter highly correlated to leaf
181 total chlorophyll content in *Acer pseudoplatanus* ($R^2=0.9295$, see Percival et al., 2008), was measured using
182 a SPAD-meter (SPAD 502. Minolta, Osaka, Japan) on the same leaves used for leaf gas exchange
183 measurements. Leaf gas exchange was measured 4, 5, 6, 15, and 17 months after the first pruning cycle, and
184 3, 5, 7, 15, and 17 months after the second pruning cycle using an infrared gas analyzer (Ciras 2, PP-System,
185 Amesbury, MA, U.S.). Measurements were conducted between 09.30 A.M. and 12.30 P.M. on the first fully

186 expanded leaf developed on marked pruned branches (21 leaves per treatment). Leaves in the cuvette were
187 provided with saturating irradiance ($1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, provided using the integrated LED light unit),
188 ambient temperature, relative humidity = 60-80% air, and CO_2 concentration = 380 ppm. Measured
189 parameters were: CO_2 assimilation (A), stomatal conductance (g_s), transpiration (E), and CO_2 concentration
190 in the substomatal chamber (C_i). Instantaneous water use efficiency (WUE) was calculated as A/E. Leaf
191 temperature (T_{leaf}) was measured using the temperature probe integrated in the Ciras cuvette.

192 CO_2 assimilation was also measured as a function of internal CO_2 concentration (A/ C_i curves). A/ C_i curves
193 were drawn by decreasing stepwise external CO_2 concentration (C_a) from 380 ppm to 30 ppm, then a C_a of
194 380 was restored and, finally, C_a was increased stepwise to 1800 ppm (Fini et al., 2014). Curves were drawn
195 3 and 7 months after the second pruning cycle (May and September, respectively). Estimates of the apparent
196 maximum rate of carboxylation by Rubisco ($V_{c,\text{max}}$) and the apparent maximum electron transport rate
197 contributing to ribulose 1,5-BP regeneration (J_{max}) were made from A/ C_i curves using the equations found by
198 Sharkey et al. (2007), as described in a previous work (Fini et al., 2011). The stomatal (L_s) and non-stomatal
199 limitations (L_{ns}) to CO_2 assimilation were calculated from A/ C_i curves as described in Lawlor (2002) and
200 Long and Bernacchi (2003). Leaf dark respiration was calculated after 20 minutes acclimation to the
201 darkness (provided by switching off the Ciras-2 integrated light source) (Ribas-Carbo et al., 2010). Then,
202 metabolic efficiency of the leaf was calculated as A/R_{dark} .

203

204 *Statistics*

205 The experimental design was a one-tree per replicate complete randomized design with seven replicates. All
206 data were analyzed with One-Way ANOVA after checking normal distribution of data using the Shapiro-
207 Wilk test (Shapiro and Wilk, 1965). Data which were not normally distributed and parameters with
208 unbalanced samples (i.e. biometrics of watersprouts) were analyzed using the non-parametric Kruskal-Wallis
209 test and means were separated using the Bonferroni test. Frequencies were calculated within each replicate
210 tree and, prior to statistical analysis, were transformed using the formula: $\arcsin \sqrt{x}$, where x is the relative
211 frequency (Amoroso et al., 2010). Differences were considered significant at $P < 0.05$ (*) and highly

212 significant at $P < 0.01$ (**). All data were analyzed using SPSS statistical software (SPSS v.20, IBM,
213 NY, U.S.).

214

215 **Results**

216 *Effect on the whole tree and wound closure*

217 Removal cuts yielded larger wounds than topping and reduction cuts, both in the first and in the second
218 pruning cycle (Table 1). Despite being small in size, wounds originated from topping cuts were the slowest
219 in closing and showed little callus and woundwood formation in the two years after pruning.

220 Before the first pruning cycle (February 2008), all trees had similar stem diameter (Table 1). Among the
221 pruning methods investigated, only topping cut depressed stem diameter growth (expressed as stem diameter
222 relative growth rate, RGR_s) compared to control trees (Table 1). RGR_s of topped trees was 21% and 34%
223 lower than for control trees after the first and second pruning cycles, respectively. On the contrary, neither
224 reduction nor removal treatments depressed stem growth as compared to control.

225 Presence of dieback on pruned branches increased due to topping (Table 1). In the first pruning cycle, the
226 only treatment to exhibit significant dieback was topping, which displayed dieback on 26% of pruned
227 branches (Table 1). For the second pruning cycle, dieback displayed for topping cuts (37%) and reduction
228 cuts (18%) were 4-fold and 2-fold more frequent than in control branches (9%).

229 70% of topped trees released root suckers, while only 40% of trees pruned with reduction cut, removal cut or
230 left unpruned released root suckers during the growing season after pruning. Furthermore, the frequency of
231 trees with more than 7 root suckers was greatly increased in topped plants compared to all other treatments
232 (Fig. 3A).

233

234 *Effect on the pruned branches: whole branch biometrics and new growth pattern*

235 All pruning techniques reduced the slenderness of the whole branch ($L:D_{wb}$) (Table 2). Removal cut
236 suppresses the whole branch, therefore $L:D_{wb}$ was not measured for this treatment. In the long term (i.e. 24
237 months after pruning), slenderness of topped and reduced branches was similar, despite topped branches

238 being less slender immediately after pruning (Table 2). During the growing season after pruning, the fast rise
239 of branch slenderness in topped trees was due to the production of very slender watersprouts in response to
240 pruning. Sprouts released after topping cuts were more slender than in all other pruning methods and had
241 similar or even higher slenderness than the unpruned shoots of control trees (Table 2). The increase in
242 slenderness of topped branches was due to increased primary growth, rather than decreased secondary
243 growth (data not shown, but primary growth of the leader shoot was on average 190% and 245% greater than
244 in reduction cut and control, respectively, after 12 months since pruning).

245 All types of pruning stimulated the release of watersprouts in the proximity of the wound, or directly from
246 the callus (Fig. 3B). In all treatments except topping, however, less than two watersprouts were developed in
247 the 20 cm proximal to the cut in over 80% of pruned branches. On the contrary, over 55% of topped
248 branches released up to 4 watersprouts and over 15% released five or more adventitious sprouts (Fig. 3B). In
249 both pruning cycles, the aspect ratio between the leader and lateral shoots/sprouts within 20 cm from pruning
250 cut was higher in those treatments (removal and topping cuts) which suppressed the apical shoot of the
251 branch without preserving (i.e. control) or substituting (i.e. reduction cut) it (Table 2).

252 The stress (σ) required to cause the failure of the attachment between the leader and the parent branch was,
253 on average, 64% and 36% lower in topped than in reduced and control branches in the first and in the second
254 pruning cycle, respectively (Table 2). On the contrary, σ in reduction cut and removal cut (the latter
255 measured only in the second cycle) did not differ from control.

256

257 *Effect on leaf characteristics and gas exchange*

258 Pruning method largely impacted leaf anatomy (Table 3). Leaves developed on topped branches were larger
259 and had lower LMA than in other pruning treatments and in control trees. In the first pruning cycle, the effect
260 of topping on leaf size and LMA was significant in the first growing season after pruning, but not in the
261 second one. As pruning was repeated, the effect of pruning method on leaf size and LMA lasted longer, and
262 leaves developed on topped branches still had larger leaf area and lower LMA even in the second growing
263 season after pruning. Leaves originated on topped branches were about 1 °C warmer than leaves of control
264 branches during the late-spring and summer period (data are the average of three measurement days

265 conducted from May to September) (Table 3). On the contrary, neither removal nor reduction treatments lead
266 to significant leaf warming compared to control.

267 Only topping cut increased significantly the leaf greenness index (Table 3). The effect was indeed transitory,
268 being only significant in the growing season immediately after pruning, then disappearing or being greatly
269 reduced the following season. Similarly, the apparent carboxylation efficiency by Rubisco ($V_{c,max}$) and the
270 apparent contribution of electron transport to Ribulose regeneration (J_{max}) were higher in the topping
271 treatment than in control during the first growing season after pruning (Table 3). Significant difference in
272 $V_{c,max}$ and J_{max} were found between these two treatments both in May and in September, 3 and 7 months after
273 pruning, respectively. On the contrary, leaves developed on reduced or removed branches had similar $V_{c,max}$
274 and J_{max} to control.

275 The effects of pruning method on CO_2 assimilation (A) were mostly restricted to the first few months
276 following pruning (i.e. 3 and 4 months after the second and the first pruning cycle, respectively) (Fig. 4A).
277 Early after pruning, only leaves of topped branches displayed higher A than control trees in both pruning
278 cycles. Later on during the growing season, differences among treatments disappeared, except on late
279 summer 2011 (17 months after the second pruning cycle), when the removal treatment displayed lower A
280 than the reduction and control treatments. Stomatal conductance (g_s) was not affected by pruning method in
281 the first pruning cycle (Fig. 4B). When pruning was repeated, an early enhancement of g_s was observed 3
282 months after pruning in the topping and reduction treatments compared to removal and control (Fig. 4B).
283 Later on in the growing season (i.e. 5 and 7 months after 2nd pruning cycle, July and September respectively),
284 g_s decreased in topped trees and increased in control, making the differences in g_s less substantial. In the
285 second growing season after the 2nd pruning cycle, leaves held on branches developed after the removal cut
286 had lower g_s than leaves of the other treatments. Intercellular CO_2 concentration (C_i) was generally decreased
287 by topping and removal cuts in the first summer after the first pruning cycle (5 and 7 months after pruning),
288 then differences were not confirmed in the second growing season (15 and 17 months after pruning) (Fig.
289 4C). As pruning was repeated, the lower C_i during summer in leaves of the topping treatment, compared to
290 control, was confirmed (5 and 7 months after the second pruning cycle), and differences were still significant
291 in the second growing season (15 and 17 months after pruning) (Fig. 4C).

292 Three months after pruning, early after full leaf expansion (May 2010), all types of pruning reduced stomatal
293 limitation to CO₂ assimilation (L_s) compared to control (Table 4). Similarly, non-stomatal limitation
294 (including mesophyll diffusion and biochemical limitations) (L_{ns}) were lower in pruned than in control
295 leaves. In detail, topping cut lead to the largest decrease in L_{ns} , whereas removal cut the least (Table 4). As
296 season progressed, and trees had to cope with stresses such as heat and reduced water availability (see the
297 change in air temperature and rainfall from May to July 2010 in fig. 1), L_s increased to a greater extent in
298 topping than in removal and reduction treatments, while control showed the lowest increase (Table 4, 7
299 months after pruning). Similarly, L_{ns} increased in all pruned treatments, but particularly in leaves of topped
300 trees which, however, yet displayed lower L_{ns} than control trees, as shown by the negative L_{ns} value.

301

302 Discussion

303 Shigo (1989) described pruning as “the best thing an arborist can do for a tree but at the same time, one of
304 the worse things an arborist can do to a tree; much depends on how pruning is carried out”. Results of this
305 experiment support Shigo’s statement by providing a quantitative evaluation of the effects of different
306 pruning methods, scaling down from the whole tree to leaf physiology and biochemistry.

307 Pruning treatments mainly differed because the apical bud of the pruned branch was suppressed (topping),
308 substituted (reduction) or retained (control), while removal cut suppressed the whole primary branch, instead
309 of its apical portion. We hypothesized that these methods may differently disturb apical dominance thereby
310 affecting subsequent growth and physiological processes and, in particular, that substituting the apical bud of
311 the branch with the one of a properly sized lateral branch through reduction cut may, at least in part, avoid
312 the complete release of apical dominance which occurs after chopping off (i.e. topping) (Hillman, 1984).
313 Results of this study clearly confirm this hypothesis.

314 First, only topped trees showed reduced stem diameter growth and increased release of root suckers
315 compared to controls. Reduction in stem diameter growth have been reported for intense pruning treatments
316 (i.e. > 50% leaf area, Pinkard and Beadle, 1998; Neilsen and Pinkard, 2003), but were unexpected here, as
317 only 30% of tree canopy was removed (Maurin and DesRochers, 2013). Because the amount of leaf area
318 removed by all pruning treatments was similar, it is unlikely that diminished stem growth of topped trees is

319 due to reduced availability of photosynthetates. Unlike other pruning methods, topping cut most likely
320 triggered the change in biomass partitioning to favour neofomed sprouts, at least partly at expenses of stem
321 growth, as reported to occur in severely defoliated trees (Hoogesteger and Karlsson, 1992; Pinkard and
322 Beadle, 1998). This is consistent with the higher emission of root suckers and watersprouts observed in
323 topped than in control trees. Enhanced release of sprouts from lateral, adventitious and latent bud has been
324 related to suppressed apical dominance (Cline, 1997). Consistently, our data show that while topping had a
325 severe effect on tree structure by greatly suppressing apical control and promoting epicormic growth,
326 pruning back a branch to a lateral with intact apical bud and large enough to become the new branch leader
327 (i.e. reduction cut) preserved normal tree growth pattern (Wilson, 2000). Similarly, removal of the whole
328 branch at its attachment to the trunk resulted in minimal disturbance to tree structure. In fact, epicormic
329 sprouts developed next to the removal pruning cut grew in the inner part of the crown (particularly after the
330 2nd pruning cycle, when trees were larger) under reduced light availability, which greatly limit their primary
331 growth and slenderness (Solomon and Blum, 1977). This is probably due to lower sink strength than the stem
332 they are attached to, resulting in photoassimilates export from the sprouts (Stoll and Schmid, 1998; Wilson,
333 2000). One of major disturbances of pruning to tree structural strength is that it inextricably leads to open
334 wounds, which may be a preferential point of entry for wood decay fungi. In this experiment, removal cuts
335 yielded larger wounds than all other treatments but, contrary to the previously reported inverse relation
336 between wound size and time of closure (Solomon and Shigo, 1976), wounds from removal cuts were the
337 fastest at closing. Not only wound size, but also the wound location within a tree, can affect wound closure
338 process. Larger wounds have been shown to lead to greater amount of discoloured wood, while poor
339 correlations are generally found between the amount of discoloured wood and closure time (Solomon and
340 Shigo, 1976; Gilman and Grabosky, 2007). It was shown, however, that the amount of wood discoloration is
341 inversely related to the vigour of the wounded plant organ, and that wound closure time is also inversely
342 related to vigour (Solomon and Blum, 1977; Armstrong et al., 1981).

343 Second, on the pruned branches, codominance of newly developed sprouts was triggered in treatments which
344 suppressed the apical axis or the whole branch, without providing a new leader. In fact, new sprouts
345 (branches in the following year) developed on topped branches and after branch removal had aspect ratio
346 higher than 0.7 whereas aspect ratios lower than 0.5 were found in reduced branches and control, as normally

347 occurs between leader and subordinate branches (Grabosky and Gilman, 2007; Gilman, 2012). We show here
348 that if the apical branch is substituted by a properly sized lateral, the latter has enough sink strength to
349 prevent extensive outgrowth from lateral buds and to maintain apical control over subordinate laterals,
350 indicating that reduction cut achieves in maintaining apical control whereas topping cut does not. From a
351 management viewpoint, codominance is one of the most hazardous structural defects of a tree which usually
352 leads to reduced tree safety, particularly if codominant branches are slender and weakly attached to the trunk
353 or the parent branch (Dahle et al., 2006; Gilman, 2012; Ciftci et al., 2013). Indeed, the stress required to
354 break the union between newly developed sprouts and their parent topped branch was about 1/3 to 2/3 lower
355 than that required to tear apart a normal branch union. This corroborates the idea that, in topped branches,
356 most of regrowth occurs from adventitious buds, which are inextricably weakly attached to the parent
357 branch, since they are attached at the cambium level (Dahle et al., 2006). Also, sprouts released in topped
358 branches were more slender than in other pruning treatments and, after the second pruning cycle, the leader
359 sprouts of topped branches were even more slender than unpruned shoots in control plants, although the
360 same was not observed for lateral sprouts. High slenderness may not be an issue for young growing axis,
361 which are flexible enough to avoid fractures even at high wind loads, which may instead cause the failure of
362 the attachment, particularly if the branch union is weak (Bertram, 1989). As branches grow old and increase
363 in size, switching from a “light-harvesting” to a structural role (which occurs when the branch is about 3 m
364 in length) slenderness starts to decline (Bertram, 1989; Dahle and Grabosky, 2010), because of reduced
365 elongation, rather than to smaller diameter growth (Dahle and Grabosky, 2010). Topping cut hinders this
366 normal ageing process of the branch by stimulating primary branch growth (long about 2.7 m just before the
367 second pruning cycle) and prevents the branch from performing a structural role. Pioneer works
368 hypothesized that removal of the apical axis may stimulate lateral axis to elongate more than they would
369 have done if the terminal had remained intact (Wilson, 1990), because of altered hormonal balance (Thimann
370 and Skoog, 1934; Prochazka and Jacobs, 1984), demonstrating that all lateral shoots have the potential to
371 become long shoots if not dominated (Suzuki and Kohno, 1987). The effects of reduction, removal and
372 topping cuts on tree hormones was not tested in this experiment; however, this knowledge would be of great
373 importance for determining best pruning practices and deserves to be addressed by future research. The fast
374 growth rate (in length) of pruned branches, the increased codominant branching and the weak branch

375 attachments in topped branches out-compassed the safety benefit resulting from the initial greater reduction
376 of whole branch slenderness immediately after pruning. Thus, despite topping appearing as a cheap and fast
377 pruning method in the short term, it has deleterious mid- and long-term effects on tree structure, thereby
378 resulting in the need of more frequent pruning and in a 4-fold rise of overall pruning cost (Campanella et al.,
379 2009).

380 Third, from the physiological viewpoint, topping stimulated vigorous resprouting from pruned branches, but
381 at the expenses of stem diameter growth and of the capacity to withstand unfavourable conditions in the
382 long-term (Harris et al., 2004; Spann et al., 2008). This may be due to the enhanced competition for light and
383 nutrients among watersprouts released from the same pruning cut. After apical control is removed by a
384 properly executed cut, the distal branch grows larger and more vertical, replaces the removed terminal and
385 restores apical control (Wilson, 2000). On the contrary, topping cut releases several co-dominant sprouts all
386 located close to the wound without any distal shoot. In this situation, becoming larger and developing larger
387 leaf area provides competitive advantage, because of higher hormone production and greater light harvesting
388 capacity compared to shorter sprouts with smaller leaf area (Wilson, 2000). Growth rate greatly depends on
389 leaf structural, biochemical and functional characteristics, with leaves with small LMA and high nitrogen
390 and chlorophyll content being commonly associated with fast-growing strategies (Reich et al., 1992; Poorter
391 and Bongers, 2006). Among pruning treatments tested here, only topping affected leaf structural traits such
392 as leaf area and leaf mass per area. The larger area of individual leaves of topped branches increased the
393 photosynthetic surface of individual branches, but resulted in leaf over-heating because of lower heat
394 dissipation by conduction/convection, than smaller leaves (Nobel, 2005). Increased leaf area in topped trees
395 was paralleled by a decrease in leaf mass per area. Leaves with low LMA are productive and often associated
396 with fast-growing plant strategies, but are necessarily short-lived and more susceptible to environmental
397 stresses (Wilson et al., 1999; Bussotti, 2008; Poorter et al., 2009). Consistently, a greater occurrence of
398 crown dieback was observed in topped trees than in other treatments after both pruning cycles.

399 Transient (lasting few weeks to few months) increases in net CO₂ assimilation (compensatory
400 photosynthesis) have been reported to occur following pruning and partial defoliation, with the magnitude of
401 this increase being positively correlated with pruning/defoliation severity (Pinkard et al., 1998; Hart et al.,

402 2000; Turnbull et al., 2007). We show here that the type of pruning, not only its severity, can modulate tree
403 responses at the leaf level. Mechanisms leading to compensatory photosynthesis are still poorly understood
404 and may involve increased stomatal conductance, increased leaf nitrogen and chlorophyll, and increase $V_{c,max}$
405 and J_{max} (Sharkey, 1985; Pinkard et al., 1998; Pinkard and Beadle, 1998; Turnbull et al., 2007). Leaf
406 structure is generally optimized for maintaining the operating $[CO_2]$ in the chloroplast stroma (C_c) at the
407 transition between the Rubisco carboxylation and RuBP regeneration limitations to photosynthesis (Farquhar
408 et al., 1980), to reduce photorespiration and, consequently, increase CO_2 assimilation (Terashima et al.,
409 2011). A tight co-regulation of stomatal and non-stomatal factors is required to achieve this goal (Flexas and
410 Medrano, 2003). Topping cut lead to an imbalance of stomatal regulation (when compared to control) which
411 was not observed in other pruning treatments. In fact, non-stomatal limitations to photosynthesis were much
412 lower in topping than in other pruning treatments and than in control, because Rubisco activity and the
413 contribution of electron transport to ribulose regeneration were greatly up-regulated in leaves developed in
414 topped branches. Higher $V_{c,max}$ and J_{max} in topping treatment resulted in transient increases in net CO_2
415 assimilation, when stomatal conductance was high enough to maintain adequate leaf internal CO_2 . Later in
416 the growing season, however, stomatal limitations increased more in leaves of topped plants than in other
417 treatments. Although leaves on topped branches still showed higher $V_{c,max}$ and J_{max} after the summer period
418 (7 months after 2nd pruning cycle), they did not show enhanced CO_2 assimilation rate compared to other
419 treatments because of high stomatal limitations, as previously hypothesized (Pinkard et al. 1998; Pinkard and
420 Beadle 1998). Maintaining higher $V_{c,max}$ and J_{max} requires large complements of enzymes and other
421 metabolites which have a substantial maintenance cost and require periodic (and costly) recycling (Reich et
422 al., 1998). Moreover, higher leaf chlorophyll and nitrogen content are commonly associated with higher
423 respiration rates (Reich et al., 1998). Consistently, the A/R_d ratio was significantly lower in leaves on topped
424 branches than in other treatments as soon as CO_2 assimilation declined because of stomatal limitation,
425 indicating that metabolic inefficiency at the leaf level is promoted by topping (Cai et al., 2009).

426 The morpho-physiological changes induced by topping were not found in plants pruned by the reduction cut,
427 suggesting that apical dominance and control may be effectively retained if the branch is pruned to a lateral,
428 large enough to become the new dominant primary axis. Removal cut, similar to topping cut, removes the
429 primary axis without proving a substitution leader. However, disturbance to plant physiology was much

430 lower, as watersprouts developed from pruning cut grow in the inner part of the canopy, and self-shading
431 resulted in a generally low photosynthetic rate, stomatal conductance and, presumably sink strength
432 (McCormick et al., 2006). This effect was clear particularly after 2nd pruning cycle, when plants were larger
433 and with broader and denser canopies, which resulted in a denser shade cast on new shoot developing from
434 the trunk.

435 In conclusion, we show here that pruning method, not only its severity, modulates the morpho-physiological
436 response of trees to pruning. Maintenance of apical control and apical dominance are key issues to preserve a
437 structurally sound tree structure, as well as the long-term efficiency of the photosynthetic apparatus. While
438 removal of the whole primary axis at its attachment to the trunk provide minimal disturbance to tree
439 physiology, shortening of the branch may provide different results, depending on where the branch is
440 shortened. Reducing the primary axis to a lateral branch large enough to become the new branch leader
441 appeared to preserve normal branching pattern and had little effects on leaf structure and photosynthetic
442 performance. On the contrary topping a branch (shortening of the primary axis without providing a
443 substitution leader) greatly affected tree structure and functioning by altering branching pattern, by
444 promoting competition among sprouts of the same branch, and by determining a shift toward a more pioneer
445 (fast growing) behaviour, but at the expense of tolerance to environmental stresses. It must be noted,
446 however, that this work dealt with young trees and further research is needed to evaluate the physiological
447 response to pruning method in mature or senescing trees.

448 **Acknowledgements**

449 This work has been done as a part of a research project called “Miglioramento delle tecniche produttive e
450 della qualità del prodotto nel vivaismo ornamentale - TECPRO” financed by Regione Lombardia –
451 Agricultural Department, according to the Plan of Research and Development 2008. Partial funding was
452 provided by UNISER Consortium, Pistoia (Italy)

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634 **Figure captions**

635 Figure 1: Monthly average temperature ($^{\circ}\text{C}$) and rainfall (mm) at the experimental site (Vertemate con
636 Minoprio, CO, Italy, $45^{\circ} 44'$ N, $9^{\circ} 04'$ E, 250 m above sea level) during the experimental period (2008 –
637 2011).

638 Figure 2: Schematic diagram of the pruning treatments imposed and of the effects of the different pruning
639 methods on new growth. The black triangles indicate that the apical bud of the branch was left untouched.
640 “Leader” and “laterals” indicate which shoots/sprouts were considered as dominant and subordinate growing
641 axes, respectively.

642 Figure 3: Frequency of: (A) number of root suckers released from the trunk flare, and (B) number of
643 watersprouts released within 20 cm from pruning cut during the first growing season after the first pruning
644 cycle. Frequencies were calculated on 7 replicate trees per treatment (root suckers) or 42 replicate pruning
645 cut per treatment (watersprouts). Different letters within the same frequency class indicate significant
646 differences among treatments at $P < 0.05$ (*) or $P < 0.01$ (**).

647 Figure 4: Effect of different pruning treatments on: A) CO_2 assimilation (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$); B) stomatal
648 conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$); and C) CO_2 concentration in the substomatal chamber (C_i , ppm) measured in
649 the 17 months after the first and the second pruning cycle. Different letter within each sampling date indicate
650 significant difference among treatments at $P < 0.05$ (*) or $P < 0.01$ (**).

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653 Table 1: Effect of different pruning methods on wound size (cm^2) and wound closure (estimated using the
654 woundwood coefficient, see method section for details), on stem relative growth rate (RGR, $\mu\text{m cm}^{-1} \text{d}^{-1}$) and
655 on the frequency of dieback on pruned branches after the first and the second pruning cycle. Stem diameter
656 measured in February 2008, right before the first pruning cycle, is also reported. Different letters within the
657 same row denote significant differences among pruning treatments at $P < 0.01$. n.d. = not determined.

	Pruning cycle	Months after pruning	Topping	Reduction	Removal	Control	P
Wound size (cm^2)	1	0	2.5 b	2.7 b	4.2 a	n.d.	0.000
	2	0	3.3 b	4.1 b	7.1 a	n.d.	0.000

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Woundwood coefficient (%)	1	12	0 b	65 a	44 b	n.d.	0.000
	1	24	10 b	93 a	72 a	n.d.	0.000
	2	12	4 b	17 a	19 a	n.d.	0.000
	2	24	24 b	43 a	50 a	n.d.	0.000
Stem diameter (cm)	1	0	6.1	6.2	6.6	6.3	0.232
RGR _{stem} ($\mu\text{m cm}^{-1} \text{d}^{-1}$)	1	0-24	8.1 b	10.8 a	10.0 a	10.3 a	0.003
	2	0-24	6.2 b	8.5 a	8.8 a	9.4 a	0.001
Crown dieback (%)	1	17	26 a	0 b	3 b	0 b	0.008
	2	17	37 a	18 b	6 c	9 c	0.005

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660 Table 2: Effects of different pruning methods on branch biometrics: slenderness of the whole branch (L:D_{wb},661 cm cm^{-1}); aspect ratio between the dominant and the subordinate shoots within 20 cm from pruning cut (cm cm^{-1});662 slenderness of the dominant shoot/sprout of the branch (L:D_{leader}, cm cm^{-1}) and of subordinate663 shoots/sprouts (L:D_{lateral}, cm cm^{-1}) and stress required to cause the failure of the union between the dominant664 shoot of the branch and the parent branch (σ , MPa). Different letters within the same row denote significant

665 differences among pruning treatments. n.d. = not determined

666

	Pruning cycle	Months after pruning	Topping	Reduction	Removal	Control	P
L:D _{wb} (cm cm^{-1})	1	0	24.2 c	35.4 b	n.d.	63.7 a	0.000
	1	12	58.5 b	64.6 b	n.d.	81.5 a	0.000
	1	24	75.8 b	75.9 b	n.d.	85.9 a	0.004
	2	0	18.3 c	57.2 b	n.d.	88.4 a	0.000
	2	12	46.8 c	64.4 b	n.d.	89.1 a	0.000
	2	24	69.9 b	71.0 b	n.d.	89.1 a	0.002
aspect ratio (cm cm^{-1})	1	12	0.86 a	0.34 b	0.82 a	0.31 b	0.000
	1	24	0.80 a	0.34 b	0.75 a	0.41 b	0.008
	2	12	0.77 a	0.29 c	0.91 a	0.47 b	0.004
	2	24	0.78 a	0.30 b	0.73 a	0.46 b	0.009
L:D _{leader} (cm cm^{-1})	1	24	94.2 a	79.4 b	60.5 c	89.9 a	0.008
	2	24	95.2 a	80.6 b	60.3 c	75.5 b	0.000
L:D _{lateral} (cm cm^{-1})	1	24	84.6 a	79.3 b	52.3 c	80.9 ab	0.015
	2	24	89.9 a	71.2 b	51.3 c	86.9 a	0.000
σ (MPa)	1	24	20.1 b	47.0 a	n.d.	53.7 a	0.020
	2	24	37.4 b	62.4 a	47.4 ab	58.5 a	0.029

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668 Table 3: Effects of different pruning methods on leaf morpho-physiological characteristics: average leaf area

669 (cm^2), leaf mass per area (LMA, g m^{-2}), leaf temperature (T_{leaf} , $^{\circ}\text{C}$), leaf greenness index, apparent rate of670 carboxylation by Rubisco ($V_{c, \text{max}}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$); apparent contribution of electron transport to ribulose671 regeneration (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), and ratio between net CO_2 assimilation and dark respiration (A/R_{dark}).672 Different letters within the same row denote significant differences among pruning treatments at $P < 0.05$.

	Pruning	Months after	Topping	Reduction	Removal	Control	P
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	cycle	pruning					
Leaf area (cm ²)	1	5	270.8 a	199.0 b	188.0 b	220.8 b	0.000
	1	17	210.1	166.2	152.5	172.1	0.683
	2	5	279.9 a	165.22 b	155.3 b	147.8 b	0.010
	2	17	183.5 a	165.9 b	155.9 b	131.6 c	0.000
LMA (g m ⁻²)	1	5	80.1 b	98.9 a	99.9 a	93.1 a	0.038
	1	17	87.3	93.3	97.8	93.8	0.817
	2	5	88.6 c	106.5 a	96.2 b	104.5 ab	0.016
	2	17	78.5 b	95.8 a	93.7 a	94.4 a	0.003
T _{leaf} (° C)	1	4-6	30.5 a	29.6 b	29.3 b	29.4 b	0.000
	1	15-17	29.7 a	29.3 b	29.0 b	29.2 b	0.000
	2	3-8	31.0 a	30.1 b	30.0 b	29.9 b	0.000
	2	15-17	29.3 a	28.4 b	28.6 b	28.3 b	0.000
Leaf greenness index (a.u.)	1	3-8	45.0 a	42.9 b	39.0 c	40.2 bc	0.000
	1	15-17	40.0	40.9	39.6	40.1	0.482
	2	3-8	42.7 a	39.1 b	35.0 c	36.9 bc	0.000
	2	15-17	39.6 a	38.3 a	35.7 b	37.3 ab	0.005
V _{c,max} (μmol m ⁻² s ⁻¹)	2	3	124.0 a	103.2 ab	93.4 b	89.5 b	0.030
	2	7	133.6 a	98.0b	93.1 b	96.0 b	0.000
J _{max} (μmol m ⁻² s ⁻¹)	2	3	226.3 a	165.5 b	141.4 b	130.2 b	0.001
	2	7	198.0 a	156.0 b	152.6 b	146.3 b	0.000
A/R _{dark}	2	3	13.06	14.34	14.11	13.08	0.754
	2	7	8.91 b	13.57 a	13.72 a	12.43 a	0.000

673

674 Table 4: Stomatal (L_s) and non-stomatal (L_{ns}) limitations to photosynthesis in leaves of *A. pseudoplatanus*
675 developed on branches subjected to different pruning methods. L_{ns} was calculated as in Lawlor (2002) and
676 Long and Bernacchi (2003) using control leaves as reference parameter. Negative L_{ns} indicates lower non-
677 stomatal limitations to CO₂ assimilation than in control trees. Different letters within the same row denote
678 significant differences among pruning treatments at P < 0.05.

	Pruning cycle	Months after pruning	Topping	Reduction	Removal	Control	P
L _s (%)	2	3	9.8 b	9.9 b	10.9 b	16.6 a	0.035
	2	7	41.0 a	21.9 b	21.9 b	18.8 b	0.015
L _{ns} (%)	2	3	-52.3 c	-16.6 b	-2.7 a	-	0.039
	2	7	-25.4 b	5.4 a	4.4 a	-	0.012

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680

- 681 • Pruning method, not only its intensity, modulates the tree response to pruning
- 682 • Reducing the apical growing axis to a lateral little disturbs branch growth
- 683 • Topping increases codominance and weakens branch structure
- 684 • In topping, higher V_{c,max} and J_{max} are not paralleled by higher CO₂ assimilation

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Figure 1

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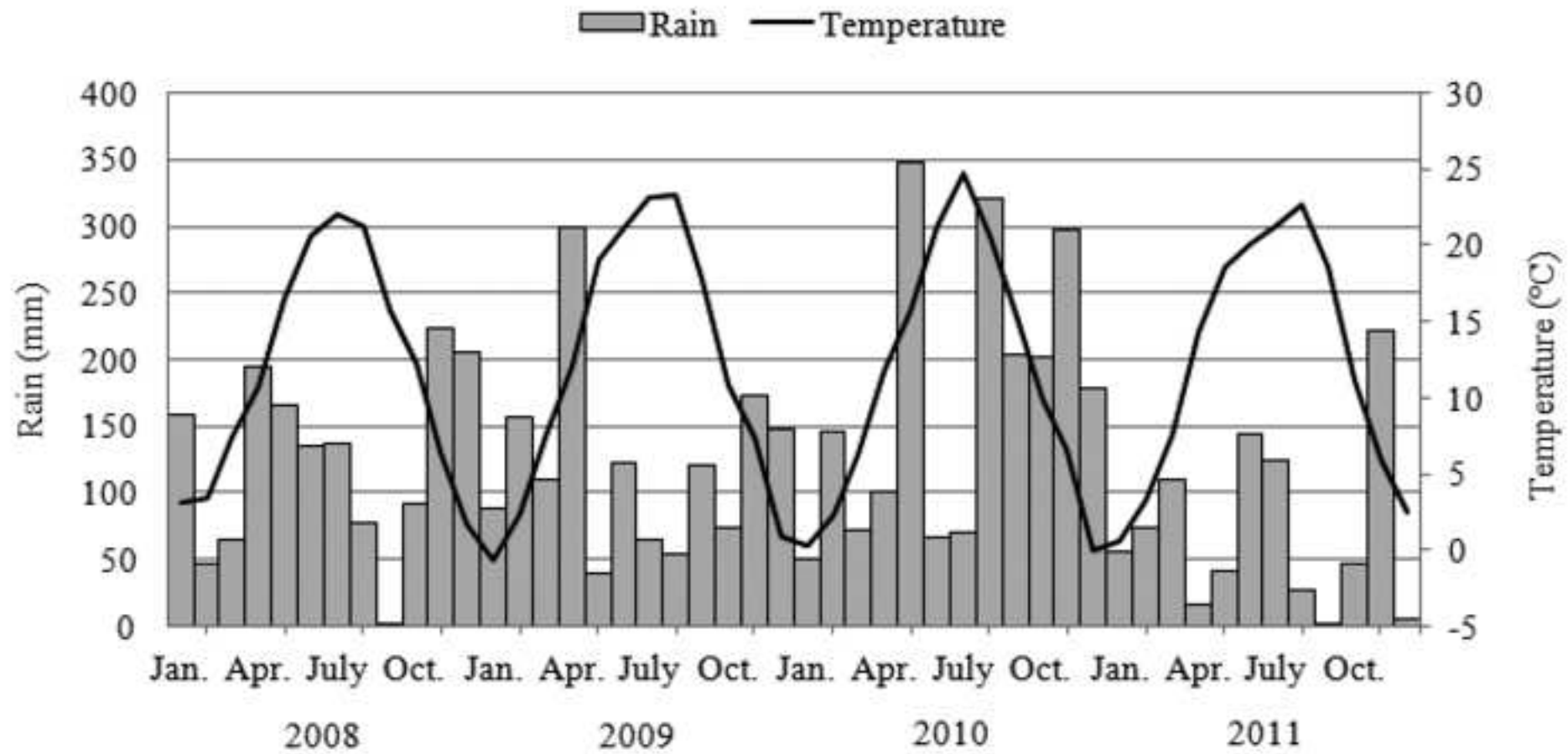
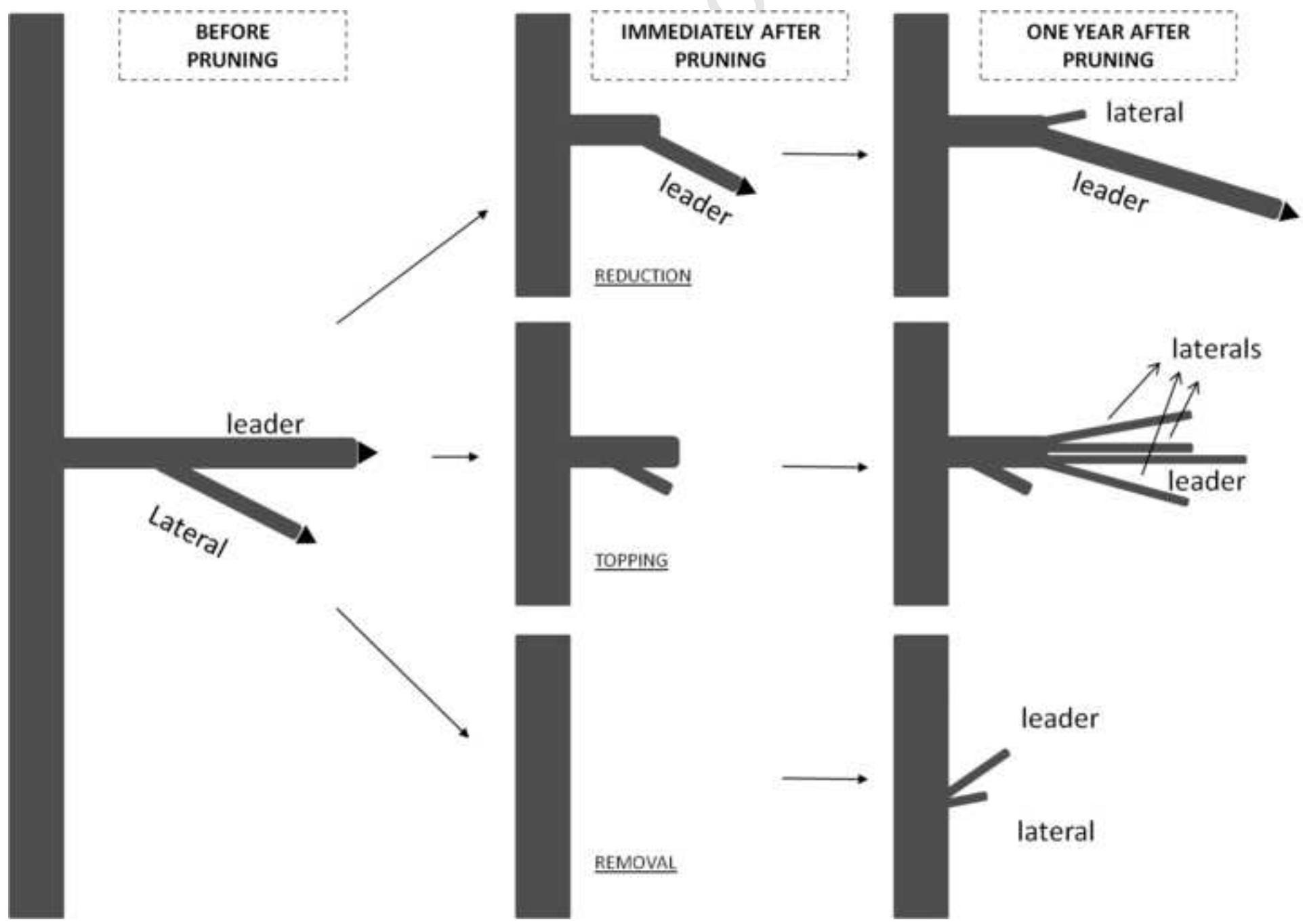


Figure 2

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