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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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4

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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 (topping) cut. Crown dieback, growth of the plant and of the pruned branches, leaf morphological traits and 20 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

(i.e. the amount of leaf area removed), modulates the morpho-physiological response of trees to pruning and
that maintenance of apical control and apical dominance are key issues to preserve a structurally sound tree
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

Trees growing in the urban environment require periodic pruning to provide clearance and improve view (i.e. trees along roadsides), to reduce conflicts with buildings and infrastructures, to thin dense canopies and decrease wind resistance, and to reduce risk of failure by removing structural defects (Dureya et al., 1996; 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 compartimentalization 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 Minoprio, Como, Italy; 45°44' N, 9°04' E), in a loamy sand, well drained soil. Mean annual rainfall in the 100 101 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 than 0.33 (Gilman, 2012); 4) Control: plants were left unpruned. In February 2010 (2nd pruning cycle), trees 116 117 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

growing season) of the newly developed sprouts was considered as the new branch leader, while theremaining 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 $[ln(\phi_{t1})$ ln $(Ø_{t0})$] * $(t_1 - t_0)^{-1}$ where: Ø is stem diameter at times 0 and 1, and $t_1 - t_0$ is time (in days) between 143 144 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.

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

(excluding, in topping and removal, the sprout designated as new leader) released from the pruning cut. Length of the leader and lateral shoots/sprouts was measured from their attachment on the higher order branch (in most cases, from the pruning cut) to the apical bud, while diameter was measured just above the attachment. The aspect ratio between the lateral and the leader was calculated as the ratio between the base 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 α / (π * d³) where: P (kN) is the maximum load; L (m) is the distance between the point of application 170 171 of the load and the attachment which was kept fixed (about 5 cm); d (m) is the inside-bark branch diameter; 172 α (rad) is the angle between the longitudinal axis of the branch and the applied load.

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 total chlorophyll content in Acer pseudoplatanus (R²=0.9295, see Percival et al., 2008), was measured using 181 182 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

expanded leaf developed on marked pruned branches (21 leaves per treatment). Leaves in the cuvette were provided with saturating irradiance (1300 μ mol m⁻² s⁻¹, provided using the integrated LED light unit), ambient temperature, relative humidity = 60-80% air, and CO₂ concentration = 380 ppm. Measured parameters were: CO₂ assimilation (A), stomatal conductance (g_s), transpiration (E), and CO₂ concentration in the substomatal chamber (C_i). Instantaneous water use efficiency (WUE) was calculated as A/E. Leaf 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, Ca 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_{e,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 CO₂ 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

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

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

- 214
- 215 Results
- 216 *Effect on the whole tree and wound closure*

Removal cuts yielded larger wounds than topping and reduction cuts, both in the first and in the secondpruning 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.

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

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

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

233

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

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

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

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

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

256

257 *Effect on leaf characteristics and gas exchange*

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

conducted from May to September) (Table 3). On the contrary, neither removal nor reduction treatments leadto 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 reduced the following season. Similarly, the apparent carboxylation efficiency by Rubisco $(V_{c,max})$ and the 269 270 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 CO₂ 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 gs was observed 3 282 months after pruning in the topping and reduction treatments compared to removal and control (Fig. 4B). Later on in the growing season (i.e. 5 and 7 months after 2nd pruning cycle, July and September respectively), 283 284 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 CO₂ 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 CO_2 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

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

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

First, only topped trees showed reduced stem diameter growth and increased release of root suckers compared to controls. Reduction in stem diameter growth have been reported for intense pruning treatments (i.e. > 50% leaf area, Pinkard and Beadle, 1998; Neilsen and Pinkard, 2003), but were unexpected here, as only 30% of tree canopy was removed (Maurin and DesRochers, 2013). Because the amount of leaf area 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 neoformed 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 2^{nd} 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).

Second, on the pruned branches, codominance of newly developed sprouts was triggered in treatments which suppressed the apical axis or the whole branch, without providing a new leader. In fact, new sprouts (branches in the following year) developed on topped branches and after branch removal had aspect ratio 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 subodrdinate 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

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

Transient (lasting few weeks to few months) increases in net CO₂ assimilation (compensatory photosynthesis) have been reported to occur following pruning and partial defoliation, with the magnitude of 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 CO₂ 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 CO₂ 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 (7 months after 2^{nd} pruning cycle), they did not show enhanced CO₂ assimilation rate compared to other 418 419 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 CO₂ assimilation declined because of stomatal limitation, 425 indicating that metabolic inefficiency at the leaf level is promoted by topping (Cai et al., 2009).

The morpho-physiological changes induced by topping were not found in plants pruned by the reduction cut, suggesting that apical dominance and control may be effectively retained if the branch is pruned to a lateral, large enough to become the new dominant primary axis. Removal cut, similar to topping cut, removes the 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.

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634 Figure captions

Figure 1: Monthly average temperature (°C) and rainfall (mm) at the experimental site (Vertemate con
Minoprio, CO, Italy, 45° 44' N, 9° 04' E, 250 m above sea level) during the experimental period (2008 –
2011).

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

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

Figure 4: Effect of different pruning treatments on: A) CO₂ assimilation (A, μ mol m⁻² s⁻¹); B) stomatal conductance (g_s, mmol m⁻² s⁻¹); and C) CO₂ concentration in the substomatal chamber (C_i, ppm) measured in the 17 months after the first and the second pruning cycle. Different letter within each sampling date indicate significant difference among treatments at P < 0.05 (*) or P < 0.01 (**).

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Table 1: Effect of different pruning methods on wound size (cm^2) and wound closure (estimated using the woundwood coefficient, see method section for details), on stem relative growth rate (RGR, $\mu m cm^{-1} d^{-1}$) and on the frequency of dieback on pruned branches after the first and the second pruning cycle. Stem diameter measured in February 2008, right before the first pruning cycle, is also reported. Different letters within the 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 | Р |
|------------|------------------|----------------------------|---------|-----------|---------|---------|-------|
| Wound size | 1 | 0 | 2.5 b | 2.7 b | 4.2 a | n.d. | 0.000 |
| (cm^2) | 2 | 0 | 3.3 b | 4.1 b | 7.1 a | n.d. | 0.000 |

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

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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 662 cm⁻¹); slenderness of the dominant shoot/sprout of the branch (L:D_{leader}, cm cm⁻¹) and of subordinate 663 shoots/sprouts (L:D_{lateral}, cm cm⁻¹) and stress required to cause the failure of the union between the dominant 664 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

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| | Pruning | Months after | Topping | Reduction | Removal | Control | Р |
|-----------------------------------|---------|--------------|---------|-----------|---------|---------|-------|
| | cycle | pruning | | | | | |
| L:D _{wb} | 1 | 0 | 24.2 c | 35.4 b | n.d. | 63.7 a | 0.000 |
| $(\mathrm{cm} \mathrm{cm}^{-1})$ | 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 | 1 | 12 | 0.86 a | 0.34 b | 0.82 a | 0.31 b | 0.000 |
| $(\mathrm{cm} \mathrm{cm}^{-1})$ | 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} | 1 | 24 | 94.2 a | 79.4 b | 60.5 c | 89.9 a | 0.008 |
| $(\mathrm{cm} \mathrm{cm}^{-1})$ | 2 | 24 | 95.2 a | 80.6 b | 60.3 c | 75.5 b | 0.000 |
| L:D _{lateral} | 1 | 24 | 84.6 a | 79.3 b | 52.3 c | 80.9 ab | 0.015 |
| $(\mathrm{cm} \mathrm{cm}^{-1})$ | 2 | 24 | 89.9 a | 71.2 b | 51.3 c | 86.9 a | 0.000 |
| σ | 1 | 24 | 20.1 b | 47.0 a | n.d. | 53.7 a | 0.020 |
| (MPa) | 2 | 24 | 37.4 b | 62.4 a | 47.4 ab | 58.5 a | 0.029 |

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

 (cm^2) , leaf mass per area (LMA, g m⁻²), leaf temperature (T_{leaf} , °C), leaf greenness index, apparent rate of

670 carboxylation by Rubisco ($V_{c, max}$, $\mu mol m^{-2} s^{-1}$); apparent contribution of electron transport to ribulose

 $\label{eq:constraint} 671 \qquad \text{regeneration} \ (J_{max}, \, \mu mol \ m^{-2} \ s^{-1}), \, \text{and ratio between net CO}_2 \ \text{assimilation and dark respiration} \ (A/R_{dark}).$

bifferent letters within the same row denote significant differences among pruning treatments at P < 0.05.

| Pruning | Months after | Topping | Reduction | Removal | Control | Р |
|---------|--------------|---------|-----------|---------|---------|---|

| | cycle | pruning | | | | | |
|---------------------------|-------|---------|---------|----------|---------|----------|-------|
| Leaf area | 1 | 5 | 270.8 a | 199.0 b | 188.0 b | 220.8 b | 0.000 |
| (cm^2) | 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 | 1 | 5 | 80.1 b | 98.9 a | 99.9 a | 93.1 a | 0.038 |
| $(g m^{-2})$ | 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} | 1 | 4-6 | 30.5 a | 29.6 b | 29.3 b | 29.4 b | 0.000 |
| (° C) | 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 | 1 | 3-8 | 45.0 a | 42.9 b | 39.0 c | 40.2 bc | 0.000 |
| greenness | 1 | 15-17 | 40.0 | 40.9 | 39.6 | 40.1 | 0.482 |
| index | 2 | 3-8 | 42.7 a | 39.1 b | 35.0 c | 36.9 bc | 0.000 |
| (a.u.) | 2 | 15-17 | 39.6 a | 38.3 a | 35.7 b | 37.3 ab | 0.005 |
| V _{c,max} | 2 | 3 | 124.0 a | 103.2 ab | 93.4 b | 89.5 b | 0.030 |
| $(\mu mol m^{-2} s^{-1})$ | 2 | 7 | 133.6 a | 98.0b | 93.1 b | 96.0 b | 0.000 |
| J _{max} | 2 | 3 | 226.3 a | 165.5 b | 141.4 b | 130.2 b | 0.001 |
| $(\mu mol m^{-2} s^{-1})$ | 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 |

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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 | Months | Topping | Reduction | Removal | Control | Р |
|--------------|---------|---------|---------|-----------|---------|---------|-------|
| | cycle | after | | | | | |
| | | pruning | | | | | |
| $L_{s}(\%)$ | 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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- Pruning method, not only its intensity, modulates the tree response to pruning
- Reducing the apical growing axis to a lateral little disturbs branch growth
- Topping increases codominance and weakens branch structure
- In topping, higher $V_{c,max}$ and J_{max} are not paralleled by higher CO_2 assimilation

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Figure 2







Figure 4

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