

**No preferential C-allocation to storage over growth in  
clipped birch and oak saplings**

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3 **1 No preferential C-allocation to storage over growth in clipped birch and oak**  
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5 **2 saplings**  
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## 23 Abstract

24 Herbivory is one of the most globally distributed disturbances affecting C-cycling in  
25 trees, yet our understanding of how it alters tree C-allocation to different functions like  
26 storage, growth or rhizodeposition is still limited. Prioritized C-allocation to storage  
27 replenishment vs. growth could explain the fast recovery of C-storage pools frequently  
28 observed in growth-reduced defoliated trees. We performed continuous  $^{13}\text{C}$ -labelling  
29 coupled to a-clipping experiment to quantify the effects of simulated browsing on the  
30 growth, leaf morphology and relative allocation of stored vs. recently assimilated C to  
31 the growth (bulk biomass) and non-structural carbohydrate (NSC) stores (soluble sugars  
32 and starch) of the different organs of two tree species ~~with contrasting wood anatomy:~~  
33 diffuse-porous (*Betula pubescens*) and ring-porous (*Quercus petraea*). C-tTransfers of  
34 C from plants to bulk and rhizosphere soil were also evaluated. Clipped birch and oak  
35 trees shifted their C-allocation patterns above-ground as a means to recover from  
36 defoliation. However, such increased allocation to current-year stems and leaves did not  
37 entail reductions in the allocation to the rhizosphere, which remained unchanged  
38 between clipped and control trees of both species. *B. pubescens* and *Q. petraea* showed  
39 differences in their vulnerability and recovery strategies to clipping, the ring-porous  
40 species being less affected in terms of growth and architecture by clipping than the  
41 diffuse-porous. These contrasting patterns could be partly explained by differences in  
42 their C cycling after clipping. Defoliated oaks showed a faster recovery of their canopy  
43 biomass, which was supported by increased allocation of recently fixed new C, but  
44 associated with large decreases in their fine root biomass. Following clipping, both  
45 species recovered NSC pools to a larger extent than growth, but the allocation of  $^{13}\text{C}$ -  
46 labelled photo-assimilates into storage compounds was not increased as compared to  
47 controls. Despite their different response to clipping, our results indicate no preventative

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3 48 allocation into storage occurred during the first year after clipping in either of the  
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5 49 species.  
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10  
11 51 **Keywords:** *Quercus petraea*, *Betula pubescens*, Carbon (C) allocation,  $\delta^{13}\text{C}$  stable  
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13 52 isotopes, non-structural carbohydrates, C-storage, below-ground allocation.  
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## 54 Introduction

55 Trees account for ca. 90% of the global biomass of carbon (C) (Körner 2003), and  
56 hence play a fundamental role in global C dynamics. C-allocation patterns in trees may  
57 shift depending on multiple factors, including age (Hartmann et al. 2018), phenology  
58 (~~Klein et al. 2016~~; Palacio et al. 2018), environmental conditions like water and light  
59 availability or temperature (Domisch et al. 2001; Messier and Nikinmaa 2000; Weber et  
60 al. 2019) and disturbances (Canham et al. 1994; ~~Raitio et al. 1994~~; Van der Heyden and  
61 Stock 1995). C-allocation within the tree biomass may determine tree vulnerability to  
62 environmental stress and disturbance (Canham et al. 1999; ~~McDowell 2011~~; ~~Myers and~~  
63 ~~Kitajima 2007~~; Wiley and Helliker 2012). Further, C-allocation to different functions  
64 like storage, growth, reproduction or rhizodeposition may affect the amount of C cycled  
65 and sequestered by trees (Hartmann et al. 2018). Understanding the response of tree C-  
66 allocation patterns to different factors may be crucial to predict the response of trees to  
67 global change (Körner 2003; Wiley and Helliker 2012).

68 Herbivory is one of the most globally distributed disturbances affecting C-  
69 cycling patterns in trees (Clark et al. 2010). Defoliation by herbivores reduces canopy  
70 leaf area causing a decrease in the net C gain of trees by current photosynthesis and  
71 altering the balance between C sinks and sources (Trumble et al. 1993). This may lead  
72 to important changes in C-allocation patterns, which can influence the environment by  
73 changes in below-ground C inputs (Eyles et al. 2009; Pinkard and Beadle 1998).  
74 Depending on the severity of damage, C demands of growing sinks may be supplied  
75 temporarily from storage (Pinkard et al. 1998; Quentin et al. 2011; Van der Heyden and  
76 Stock 1995), namely non-structural carbohydrates (NSC) and lipids, some of which can  
77 be mobilised to support growth or other plant functions (Chapin et al. 1990).  
78 Accordingly, several studies have reported a decrease in starch pools after defoliation in

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3 79 deciduous (Canham et al. 1994; Kosola et al. 2001; Van der Heyden and Stock 1995)  
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5 80 and evergreen tree species (Ericsson et al. 1985; Fierravanti et al. 2019; ~~Webb and~~  
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7 ~~Karehesy 1977~~). However, trees are able to compensate to some degree for loss of  
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9 81 foliage by changing allocation patterns (e.g. favouring foliage production), upregulating  
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11 82 photosynthesis and changing leaf morphology (Fuenzalida et al. 2019; ~~Hoogesteger and~~  
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13 83 ~~Karlsson 1992; Pinkard et al. 2007~~; Pinkard and Beadle 1998). Recovery from light  
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15 84 defoliation is considered to rely mainly on current photo-assimilates produced by  
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17 85 surviving foliage (Barry et al. 2011) and does not normally cause a significant decrease  
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19 86 in NSC pools (~~Piper and Fajardo 2014~~; Tschaplinski and Blake 1994; Van der Heyden  
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21 87 and Stock 1995). In the case of moderate or severe defoliation, decreases in NSC  
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23 88 concentrations tend to be transient and of short duration, becoming non-significant over  
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25 89 the course of a growing season, while the effects on tree growth seem to be more long-  
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27 90 lasting (Palacio et al. 2008; Piper et al. 2015; Puri et al. 2015). Sometimes defoliated  
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29 91 trees show even higher NSC concentrations than undefoliated controls (Palacio et al.  
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31 92 2012; Piper et al. 2015; ~~Ramirez et al. 2018~~).

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37 94 The differential dynamics of growth vs. recovery of NSC pools in defoliated  
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39 95 trees have been interpreted in relation to two, non-exclusive processes: 1) a C-sink  
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41 96 limitation to growth due to reductions in the numbers of buds, limiting levels of non-C  
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43 97 reserves, hormonal changes or allometric adjustments in response to reduced leaf area,  
44  
45 98 leading to surplus-C being allocated to storage (Palacio et al. 2012; Palacio et al. 2008;  
46  
47 99 Piper et al. 2015; Puri et al. 2015; Schmid et al. 2017); 2) a preventative prioritized C-  
48  
49 100 allocation to storage over growth, ultimately leading to C-limitation (Piper et al. 2015;  
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51 101 Puri et al. 2015; Wiley et al. 2017a; Wiley and Helliker 2012; Wiley et al. 2013).  
52  
53 102 Preferential allocation of C to NSC over growth has recently been demonstrated in C-  
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55 103 starved plants subjected to prolonged shading (Weber et al. 2019) or complete darkness  
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3 104 (Weber et al. 2018). Nevertheless, its occurrence in other potentially C-limiting  
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5 105 conditions such as defoliation remains equivocal (Wiley et al. 2017a; ~~Wiley et al. 2013~~).

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8 106 In addition to changes in C-allocation among tree organs, defoliation can induce  
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10 107 shifts in C-transfers below-ground, influencing soil microbial communities (Bardgett  
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12 108 and Wardle 2003; ~~Pestaña and Santolamazza-Carbone 2011~~, but see Barto and Rillig  
13  
14 109 2010) and nutrient cycling (Ayres et al. 2004). Approximately 50% of the C produced  
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16 110 by woody plants is allocated below-ground, either directly to the roots, or as  
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18 111 rhizodeposition of C exudates from roots to the surrounding soil (Giardina et al. 2005).  
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20 112 Defoliation can reduce below-ground C-allocation by enhancing fine root mortality,  
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22 113 particularly in trees (Bryant et al. 1993; Tuomi et al. 1990; Vanderklein and Reich 1999,  
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24 114 but see Endrulat et al. 2016; Kosola et al. 2001). In contrast, in herbaceous plants  
25  
26 115 herbivory can increase short-term allocation of C below-ground (Orians et al. 2011), as  
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28 116 has also been found for *Populus* spp. (Babst et al. 2005). Defoliation has been  
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30 117 demonstrated to elicit short-term increases in the flux of C to root exudates in grasses  
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32 118 (Paterson et al. 2005), while the detection of effects in woody species remains elusive  
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34 119 (Ayres et al. 2004; Frost and Hunter 2008). In general, there is a lack of information  
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36 120 related to below-ground responses of woody plants to defoliation.  
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42 121 Differences in wood anatomy have also been assumed to entail differences in C-  
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44 122 allocation dynamics (Barbaroux and Bréda 2002), with putative consequences on the  
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46 123 response of trees to defoliation (Foster 2017). Ring-porous species complete part of  
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48 124 earlywood growth (including large earlywood vessel formation) before bud burst in  
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50 125 spring (Dougherty et al. 1979). This phenology is putatively a result of winter embolism  
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52 126 of large-diameter vessels, and the need to produce a new set of xylem vessels prior to  
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54 127 bud burst to supply newly emerging leaves with water (Lechowicz 1984). Contrastingly,  
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56 128 diffuse-porous species have only small xylem vessels and winter embolism has  
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3 129 relatively less impact on the hydraulic conductivity of the tree, so leaf expansion can  
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5 130 proceed using ~~xylem formed in the previous growing season~~ established xylem, without  
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8 131 the need to produce new radial stem growth (~~Essiama and Eschrich 1985~~; Lechowicz  
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10 132 1984). The consequence for C-cycling is that ring-porous species show greater seasonal  
11  
12 133 variations in NSC pools and concentrations, and a relatively greater dependence on the  
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14 134 remobilization of stored NSC for earlywood growth in spring, than diffuse-porous  
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16 135 species (Barbaroux and Bréda 2002; Barbaroux et al. 2003, but see Palacio et al. 2011).  
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18 136 It has recently been suggested that these differences in C storage and allocation  
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20 137 underline potential differences between ring-porous and diffuse-porous species in the  
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22 138 vulnerability to spring defoliation, the former being more resistant to defoliation owing  
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24 139 to their larger C-stores and advanced wood growth phenology (Foster 2017).  
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28 140 Nevertheless, to our knowledge this possibility has never been explored experimentally.

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31 141 We performed continuous  $^{13}\text{C}$ -labelling coupled to a clipping experiment to  
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33 142 quantify the effects of simulated browsing on the relative allocation of stored and  
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35 143 recently assimilated C to growth (bulk biomass) and NSC (soluble sugars and starch) of  
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37 144 the different organs of two tree species with contrasting wood anatomy: diffuse-porous  
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39 145 downy birch (*Betula pubescens* Ehrh.) and ring-porous sessile oak (*Quercus petraea*  
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41 [Matt.] Liebl.). Transfers from the plants to bulk (i.e. not in direct contact with tree  
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43 146 roots) and rhizosphere soil were also evaluated. Clipping was selected ~~as a defoliating~~  
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45 147 ~~treatment~~ to mimic the effects of browsing: a major factor hampering the regeneration  
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47 148 of native forests worldwide (~~Côté et al. 2004~~; Gill 2006; Hester et al. 2004). The use of  
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49 149 continuous  $^{13}\text{C}$ -labelling at close-to-ambient concentrations was chosen as a quantitative  
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51 150 mean to separate current from stored C-assimilates, estimate C-allocation to different  
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53 151 organs and C-compounds over the course of the growing season and track allocation  
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55 152 below-ground, without the potential drawbacks of pulse-chase labelling (see Paterson et  
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3 154 al. 2009). We hypothesized that: ~~(i)~~ clipping would lead to: (i) increased C-allocation  
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5 155 above-ground vs. below-ground (i.e. roots and the rhizosphere); (ii) ~~clipped trees would~~  
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7 156 ~~show~~ increased allocation of new C into storage ~~relative to control trees~~; and (iii)  
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9 157 ~~detrimental effects of clipping on reduced~~ tree-growth and C storage ~~would be~~ more  
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12 158 noticeable in birch than in oak, owing to the ring-porous wood anatomy and subsequent  
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15 159 larger storage C-pools of the latter (Foster 2017).  
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## 19 161 **Materials and methods**

### 20 162 *Experimental set up*

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22 163 The experimental set up was the same as described in Palacio et al. (2011). In brief, in  
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24 164 2007 we applied two clipping treatments: control (unclipped) and clipped (i.e. 66%  
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26 165 shoots removal in two consecutive dates: July and September 2007) to two-year-old  
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28 166 sessile oak (*Quercus petraea* [Matt.] Liebl.) and downy birch (*Betula pubescens* Ehrh.)  
29  
30 167 saplings planted in pots (Fig. 1). In April 2008, before bud burst, five trees of each  
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32 168 species and treatment were harvested to account for differences in biomass and NSC  
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34 169 allocation in the short-term. At that same time, five extra trees of each species and  
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36 170 treatment were moved into a polytunnel with altered  $\delta^{13}\text{C}$  air composition to take part in  
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38 171 a continuous  $\delta^{13}\text{C}$ -labelling experiment, while five control trees of each species were  
39  
40 172 left at the greenhouse to serve as “ambient” trees. The aim of the continuous labelling  
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42 173 was to separate newly fixed C from “old” C. In August 2008, trees from the C-labelling  
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44 174 experiment were harvested to evaluate differences in new C allocation to bulk biomass  
45  
46 175 and NSC (SS and starch) between clipped and control trees of both species one year  
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48 176 after clipping (Fig. 1). “Ambient” trees were harvested in November 2008 to provide  
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50 177 natural abundance  $\delta^{13}\text{C}$  values of the different organs of both species to be used in  
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52 178 calculations. Further details of these experimental procedures follow.  
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180 *Clipping experiment*

181 Trees of both study species were lifted from a nursery while dormant (5 April 2007) and  
182 planted in 44 l pots filled with gravel at the bottom for drainage and freely-drained soil  
183 derived from granite and granitic gneiss (Countesswells Association, Glentworth and  
184 Muir 1963). At planting, saplings were approximately 0.3-0.5 m high, a stage  
185 considered highly vulnerable to large herbivore browsing in nature (Gill 2006; Hester et  
186 al. 2000; Hester et al. 1996). After planting, saplings were moved into an unheated  
187 greenhouse and 20 trees of each species were randomly allocated into “clipped” and  
188 “control” treatments, leading to 10 replicates per species and treatment combination.  
189 Five extra trees per species were allocated to the “ambient” group, which did not  
190 receive clipping or  $^{13}\text{C}$ -labelling. Trees were numbered and positioned in the  
191 greenhouse following a Latin square design. Between April and November 2007, soil  
192 was kept moist with tap water without exceeding field capacity and saplings received  
193 0.5 l of a nutrient solution with  $3.0 \text{ mol N m}^{-3}$  as  $\text{NH}_4\text{NO}_3$ ,  $1.33 \text{ mol m}^{-3} \text{ Na}_2\text{HPO}_4 \cdot 12$   
194  $\text{H}_2\text{O}$  and  $1 \text{ mol m}^{-3} \text{ K}_2\text{SO}_4$  once per week, to remove any potential nutrient limitation to  
195 growth. A natural photoperiod was used and the greenhouse ventilated to provide  
196 temperatures close to ambient. To account for initial tree variability and avoid potential  
197 confounding effects on tree growth, morphological measurements (tree height, length,  
198 stem diameter, and number of short shoots and long shoots) were taken from every tree  
199 at planting and prior to each clipping and harvest.

200 Clipping was applied as in Palacio et al. (2011) by removing 66% of current-  
201 year shoots (2 out of every 3 current-year shoots) in early July and early September  
202 2007, after the first and second flushes of shoot growth were finished (Fig. 1). This  
203 intensity of damage was selected to reproduce high densities of browsing animals

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3 204 (Speed et al. 2011). In birch, clipping treatments were designed to reproduce browsing  
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5 205 damage by red deer or sheep by removing current-year long-shoots (including stems,  
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7 206 buds and leaves) up to the maximum stem diameters normally eaten by red deer or  
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10 207 sheep (Shipley et al. 1999). In oaks, clipped shoots were selected to ensure a decrease in  
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12 208 total tree leaf area of approx. 66% owing to the highly variable shoot length and fewer  
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14 209 shoots of this species. While the use of clipping to simulate browsing has received some  
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16 210 criticism (Baldwin 1990), woody plant responses to well-simulated damage do not  
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18 211 differ significantly from responses to real herbivore damage (Bergman 2002; Hester et  
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20 212 al. 2004).

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26 214 *Short-term effects of clipping on biomass and carbohydrate allocation*

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28 215 Twenty dormant saplings (five of each species and clipping treatment) were removed  
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30 216 from their pots on 2 April 2008 and separated into: one and two-year-old stems (i.e.  
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32 217 stems formed in 2007 and 2006, respectively), woody stems (>2 years), coarse roots (>  
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34 218 2 mm diameter) and fine roots (< 2mm diameter). Samples were freeze-dried and  
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36 219 weighed (*ca.* 0.005 mg) and then milled to a fine powder in a ball mill (Retsch Mixer  
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38 220 MM301, Leeds, UK).

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42 221 Soluble sugars (SS) were extracted with 80% (v/v) ethanol and their  
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44 222 concentration determined using the phenol-sulphuric method as modified by Buysse and  
45  
46 223 Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after  
47  
48 224 ethanol extractions were reduced (i.e. enzymatically) to glucose and analyzed as  
49  
50 225 described in Palacio et al. (2007).

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56 227 *Continuous <sup>13</sup>C labelling experiment*

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3 228 On 1 April 2008, five saplings from each species and clipping treatment combination  
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5 229 were transferred to an aluminium and polythene tunnel, 2.4 m wide, 3.0 m long and 2.2  
6  
7 230 high (Super 8 Hobby Tunnel, Northernpolytunnels, Colne, UK) as described previously  
8  
9 231 (Palacio et al., 2011; Fig. 1). At this time, trees were only just starting to break bud. The  
10  
11 232 polytunnel was supplied with air having CO<sub>2</sub> with a depleted <sup>13</sup>C-signature (relative to  
12  
13 233 atmospheric CO<sub>2</sub>), in order to differentiate current (new) from previous (old) plant  
14  
15 234 assimilates (Nogués et al. 2004; Paterson et al. 2009). This was achieved by partially  
16  
17 235 scrubbing CO<sub>2</sub> from the air using a CO<sub>2</sub>-scrubber unit (Texol, Dundee, UK) that  
18  
19 236 reduced the CO<sub>2</sub> concentration to 74-103 μmol mol<sup>-1</sup>. The scrubbed air was then mixed  
20  
21 237 with CO<sub>2</sub> from a gas cylinder (BOC, Worsley, UK) with a δ<sup>13</sup>C-signature of -34.0 ‰,  
22  
23 238 using Brooks 580s thermal mass flow controllers, interfaced with a Brooks control unit  
24  
25 239 (both Flotech Solutions Ltd, Stockport, UK). Resulting CO<sub>2</sub> concentrations inside the  
26  
27 240 polytunnel averaged 332 ppm and had an average δ<sup>13</sup>C of -21.4‰. Temperature inside  
28  
29 241 the polytunnel was checked regularly with a shielded thermometer. On the 6<sup>th</sup> of May  
30  
31 242 2008 we installed a shade mesh intercepting ~30% of the light on top of the polytunnel  
32  
33 243 to reduce warming.

34  
35 244 Trees were distributed within the polytunnel following a Latin square design,  
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37 245 which was changed in the middle of the experiment. They regularly received 0.5 l of the  
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39 246 same nutrient solution described above. We took initial and final tree morphological  
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41 247 measurements (as described above) at the beginning and at the end of the <sup>13</sup>C-labelling  
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43 248 period. On 5th August 2008, four months after the beginning of the <sup>13</sup>C-labelling, trees  
44  
45 249 inside the polytunnel were harvested for analysis (Fig. 1). At this time, leaf senescence  
46  
47 250 was starting and most of the annual growth had been completed. Harvested trees were  
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49 251 separated into: current-year (formed in 2008), one and two-year-old stems (formed in  
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51 252 2007 and 2006, respectively), woody stems (>2 years), coarse roots (> 2mm diameter)  
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3 253 and fine roots (< 2mm diameter). A three-year-old branch was clipped off each tree to  
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5 254 measure leaf area, individual leaf weight and specific leaf area according to the  
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7 255 protocols in Cornelissen et al. (2003). Rhizosphere and bulk soil were harvested from  
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10 256 each pot. Rhizosphere soil was collected by separating roots from the soil, gently  
11  
12 257 shaking them and then submerging fine roots in distilled water. Samples were freeze-  
13  
14 258 dried and weighed (ca. 0.005 mg). Samples were milled to a fine powder in a ball mill  
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16  
17 259 (Retsch Mixer MM301, Leeds, UK) and analysed for NSC as described above.  
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### 261 *Ambient trees*

262 In early November 2008, the five non-clipped, "ambient", saplings of each species were  
263 harvested from the greenhouse for analysis of the  $\delta^{13}\text{C}$  at natural abundance in the bulk  
264 biomass of the same fractions considered for  $^{13}\text{C}$ -labelled trees. Throughout 2008,  
265 growth conditions for these trees were similar to those in 2007 (see above). At the time  
266 of harvest, trees were shedding their leaves and radial growth had been completed.

### 268 *C isotope analysis*

269 The  $^{13}\text{C}$  signature of samples was measured by continuous flow isotope ratio mass  
270 spectrometer (Thermo Finnigan Delta Plus<sup>advantage</sup>) interfaced to an elemental analyser  
271 (Thermo FlashEA1112, Thermo Finnigan, Bremen, Germany). Data were expressed as  
272  $\delta^{13}\text{C}_{\text{V-PDB}}$ :

$$273 \quad \delta^{13}\text{C} (\text{‰}) = (R_{\text{S}} / R_{\text{VPDB}} - 1) \times 1000$$

274 where  $R_{\text{S}}$  and  $R_{\text{VPDB}}$  are the molecular abundance ratios of carbon isotopes ( $^{13}\text{C}/^{12}\text{C}$ ), of  
275 the sample and international standard (Vienna Pee Dee Belemnite), respectively. Long-  
276 term precision for quality control standards (milled flour) was  $\delta^{13}\text{C}_{\text{V-PDB}}$ :  $-26.0 \pm$   
277  $0.24\text{‰}$  (mean  $\pm$  SD,  $n=187$ ).  $\delta^{13}\text{C}$  was measured in bulk plant biomass, bulk soil and

278 rhizosphere soil. Measurements of  $\delta^{13}\text{C}$  in SS and starch followed compound-specific  
 279 analyses as detailed below.

280  $\delta^{13}\text{C}$  in NSC were measured following the procedure in Tcherkez et al. (2003).  
 281 In brief, leaf powder was suspended with 1 mL of distilled water in an Eppendorf tube  
 282 (Eppendorf Scientific, Hamburg, Germany). After centrifugation, starch was extracted  
 283 from the pellet by HCl solubilization. Soluble proteins of the supernatant were heat  
 284 denatured and precipitated and SS and organic acids of the protein-free extract were  
 285 separated by HPLC. After lyophilization, 200 mg of purified starch were weighted into  
 286 tin capsules (Courtage Analyze Service, Mont Saint-Aignan, France) for isotope  
 287 analysis. Determinations of  $\delta^{13}\text{C}$  in NSC were conducted at the *Centres Científics i*  
 288 *Tecnològics* (CCiT) of the University of Barcelona using an elemental analyser  
 289 (EA1108, Series 1, Carbo Erba Instrumentazione, Milan, Italy) coupled to an isotope  
 290 ratio mass spectrometer (Delta C, Finnigan, Mat., Bremen, Germany) operating in  
 291 continuous flow mode. Data were expressed as indicated above.

### 293 *Calculations*

294 The proportion of newly assimilated C in the bulk biomass, SS and starch of the  
 295 different fractions of trees grown in  $^{13}\text{C}$ -depleted conditions ( $F_{\text{Cnew}}$ ) was calculated  
 296 using the following equation (Nogués et al. 2004):

$$297 \quad F_{\text{Cnew}} = \frac{\delta^{13}\text{C}_{\text{Sample}} - \delta^{13}\text{C}_{\text{Ambient}}}{\delta^{13}\text{C}_{\text{Grass}} - \delta^{13}\text{C}_{\text{Ambient}}}$$

298 Where  $\delta^{13}\text{C}_{\text{Sample}}$  was the isotopic composition in the sample;  $\delta^{13}\text{C}_{\text{Ambient}}$  was the natural  
 299 baseline  $\delta^{13}\text{C}$  value for the bulk biomass, SS or starch of a given fraction of each species  
 300 calculated from fractions collected from ambient trees in November 2008 (in the case of  
 301 the bulk biomass) or, in the case of SS and starch, by applying an enrichment of 1.71  
 302 and 2.02, respectively, owing to values reported in the literature (Badeck et al. 2005);

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3 303 and  $\delta^{13}\text{C}_{\text{Grass}}$  was the average of the  $\delta^{13}\text{C}$  values obtained for *Lolium perenne* plants that  
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5 304 were grown from seed within the polytunnel and hence represented C arising from  
6  
7 305 current assimilation. We assumed that the discrimination against  $^{13}\text{C}$  during  
8  
9 306 photosynthesis would be the same in both the grasses and the trees, because the grasses  
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11 307 were grown under the same conditions as the trees and had no water stress.  
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### 309 *Statistical analyses*

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19 310 We used univariate general linear models (glm) to analyse for differences in biomass  
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21 311 and NSC-allocation between species, fractions and treatments. Short-term effects of  
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23 312 simulated browsing on biomass and NSC-allocation of trees harvested in April 2008  
24  
25 313 (before the  $^{13}\text{C}$ -labelling experiment) and the effects of simulated browsing on tree  
26  
27 314 growth, architecture, leaf morphology, biomass, NSC and % new C-allocation (in bulk  
28  
29 315 biomass, SS and starch) and NSC concentrations in the year after clipping (trees  
30  
31 316 harvested in August 2008) were evaluated by glms with species, fractions and  
32  
33 317 treatments as fixed effects. Treatment effects were further tested within fractions and  
34  
35 318 species using one-way ANOVAs. The initial length (i.e. distance from the base to the  
36  
37 319 tip of the tree) of trees at the beginning of the experiment was included as a covariate in  
38  
39 320 all analyses. Starch and NSC concentrations were angularly transformed to meet  
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41 321 normality and homogeneity in variance assumptions. Analyses were done with SPSS  
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43 322 Statistics 17.0.  
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## 324 **Results**

### 325 *Short-term effects of clipping on biomass and NSC allocation*

326 At the beginning of the labelling experiment (i.e. seven months after the application of  
327 treatments), trees subjected to two successive clipping events were significantly smaller  
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3 328 than control trees and showed a significant reduction in the biomass of the youngest  
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5 329 shoot cohort, i.e. that formed in 2007 and directly affected by clipping, but larger main  
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7 330 stem biomass (particularly in birch, Table 1, Table S1 available as Supplementary Data  
8  
9 331 at Tree Physiology Online). Trees were still dormant and leafless at this time, and no  
10  
11 332 other significant differences were observed in the allocation to different tree fractions in  
12  
13 333 either of the two species. Differences in allocation to different plant components were  
14  
15 334 quantified between birch and oak trees: birch allocated significantly more biomass to  
16  
17 335 above-ground fractions like young and main stems, while oak had significantly more  
18  
19 336 biomass in coarse roots (Table 1, Table S1). However, no significant treatment x  
20  
21 337 species interaction was found, indicating that clipping did not lead to a different  
22  
23 338 response in biomass allocation between species in the short-term.  
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28 339 Clipped trees of both species harvested in April 2008, seven months after the  
29  
30 340 application of treatments, showed significantly higher starch concentrations in young  
31  
32 341 stems (Tables S1 and S2, available as Supplementary Data at Tree Physiology Online).  
33  
34 342 However, NSC pools were decreased in the youngest shoot cohort, showing the  
35  
36 343 significant effect of clipping on the biomass reduction of this cohort for both species  
37  
38 344 (Tables S1 and S3, available as Supplementary Data at Tree Physiology Online). Starch  
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40 345 and SS pools in main stems were significantly larger in clipped trees of both species  
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42 346 (Tables S1 and S3, available as Supplementary Data at Tree Physiology Online). This  
43  
44 347 was not due to increased NSC concentrations (Tables S1 and S2, available as  
45  
46 348 Supplementary Data at Tree Physiology Online), but to higher allocation of biomass to  
47  
48 349 main stems in clipped trees (Tables 1 and S1 available as Supplementary Data at Tree  
49  
50 350 Physiology Online). Overall, birch trees had higher SS concentrations, while oaks  
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52 351 showed up to three times higher starch concentrations, particularly in coarse roots  
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54 352 (Tables S1 and S2 available as Supplementary Data at Tree Physiology Online).  
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5 354 *Changes in growth, architecture, leaf morphology, biomass and NSC allocation one*  
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8 355 *year after clipping*  
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10 356 Measurements taken at the end of the first year after the application of clipping  
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12 357 treatments (trees harvested in August 2008), indicated a lower compensating ability in  
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14 358 birch than in oak (Table 2). Although the effect of clipping on tree height was not  
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16 359 significant in the general model ( $F = 2.5$ ,  $P = 0.134$ ), significant treatment effects arose  
17  
18 360 in birch when both species were analysed separately, with clipped trees being  
19  
20 361 significantly shorter than controls (Table 2). Clipped *B. pubescens* trees also had fewer  
21  
22 362 branches, terminal shoots and short shoots than controls, indicating that significant  
23  
24 363 effects on the architecture of clipped trees remained measureable even one year after  
25  
26 364 clipping. On the contrary, regrowth of *Q. petraea* saplings completely compensated for  
27  
28 365 height and branching differences, with clipped trees showing only a marginally  
29  
30 366 significant smaller number of terminal shoots than control trees after one year (Table 2).  
31  
32 367 As regards the morphology of leaves, we observed no significant differences in  
33  
34 368 individual leaf area and weight or the SLA of clipped and control trees of both species  
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36 369 (Table 2).

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42 370 There were no significant differences in biomass allocation to leaves and coarse  
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44 371 roots of both species in trees harvested one year after clipping, but clipped trees of both  
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46 372 birch and oak allocated significantly more biomass to current-year stems than control  
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48 373 trees (Fig. 2). In oak, clipped trees showed more biomass allocation to the main stems  
49  
50 374 and a sharp reduction in biomass allocation to fine roots, which were reduced by 45% in  
51  
52 375 relation to control trees (Fig. 2). Such reduction in fine root biomass led to a significant  
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54 376 decrease in the root:shoot ratio of clipped oaks, not observed in birch (Table 2). At the  
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56 377 end of the first growing season after clipping, the significant reduction of shoots formed  
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3 378 in 2007 observed at the beginning of the C-labelling experiment in both species (Table  
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5 379 1) was only noticeable in birch, and differences between treatments in oak were no  
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8 380 longer significant (Fig. 2). There were no significant differences in total plant biomass  
9  
10 381 between treatments, either in the general model ( $F = 3.1$ ,  $P = 0.098$ ) or when species  
11  
12 382 were analysed separately ( $F = 2.3$ ,  $P = 0.169$  and  $F = 1.3$ ,  $P = 0.280$  in birch and oak,  
13  
14 383 respectively).

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17 384 Differences between control and clipped trees in NSC pools of different plant  
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19 385 fractions mimicked results for biomass allocation. Higher SS (and also starch in the case  
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21 386 of oak) pools were found in current-year stems of clipped trees of both species (Fig. 3).  
22  
23 387 Contrastingly, NSC pools were lower in one-year old stems of clipped birch trees (Fig.  
24  
25 388 3). Clipped oak trees showed also a trend for higher net allocation of SS to main stems,  
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27 389 while both starch and SS pools were significantly reduced in their fine roots (Fig. 3).  
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30 390 The differences observed in NSC pool allocation were not driven by changes in  
31  
32 391 NSC concentrations, which remained similar for both SS and starch across treatments  
33  
34 392 and fractions, except for SS concentrations in the leaves of clipped birch trees, which  
35  
36 393 were lower than those of control trees (Table 3). In any case, the lower SS  
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38 394 concentrations of the leaves of clipped birch trees did not result in significantly different  
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40 395 SS pools between control and clipped birch trees (Fig. 3, Table 3).  
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47 397 *Changes in the net allocation of newly fixed C to different plant fractions and the soil*  
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49 398 *during the recovery of clipped birch and oak trees*

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51 399 The two species showed very different responses in their  $\delta^{13}\text{C}$  isotopic composition  
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53 400 after labelling and clipping (Table S4 available as Supplementary Data at Tree  
54  
55 401 Physiology Online). In general, birch allocated significantly more new C to biomass  
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57 402 than oak, pointing to a lower reliance on C-stores and a higher C-fixing ability of birch  
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3 403 than oak (Fig. 4, Table 4). Across species and treatments, the fractions receiving  
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5 404 proportionally more newly fixed C were rapidly growing ones, including, in descending  
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7 405 order, leaves, current-year stems, young (one and two-year-old) stems and fine roots  
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9 406 (Fig. 4). Despite being winter deciduous, the leaves of both species were mainly built on  
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11 407 newly fixed C, which accounted for 80% of the bulk leaf biomass in birch and over 65%  
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13 408 in oak (Fig. 4).

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17 409 Clipped trees allocated significantly more newly-fixed C to the different  
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19 410 fractions than controls (Table 4, Fig. 4), but results were very different depending on the  
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21 411 species (Fig. 4, Table S5 available as Supplementary Data at Tree Physiology Online).  
22  
23 412 This explains why all interaction terms in the general model were significant (Table 4).  
24  
25 413 In general, clipped trees tended to allocate more new C into fast growing fractions like  
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27 414 young stems and fine roots than control trees (Fig. 4, Table S5 available as  
28  
29 415 Supplementary Data at Tree Physiology Online), but when both species were analysed  
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31 416 separately, effects were only significant in oak (Fig. 4). In the year after clipping,  
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33 417 clipped *Q. petraea* trees allocated significantly more new C than controls to all fractions  
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35 418 but the main stems (Fig. 4). Contrastingly, birch trees showed no differences in the  
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37 419 allocation of new C to different fractions between control and clipped trees, except for  
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39 420 the main stems, where control trees received a larger proportion of new C (Fig. 4).

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44 421 The rhizosphere soil collected underneath both study species showed more  $^{13}\text{C}$ -  
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46 422 depleted values as compared to the bulk soil ( $F = 118.6$ ,  $P < 0.001$ ; Fig. 5), indicating  
47  
48 423 that plant roots significantly altered the  $\delta^{13}\text{C}$  signature of the soil in direct contact with  
49  
50 424 them.  $\delta^{13}\text{C}$  values of the rhizosphere soil collected underneath birch trees were more  
51  
52 425 depleted than those of oak, potentially indicating a larger amount of labelled-C  
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54 426 transferred to the rhizosphere in this species ( $F = 6.5$ ,  $P = 0.016$ ; Fig. 5). However, no  
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56 427 significant clipping effects were observed in the  $\delta^{13}\text{C}$  signature of bulk and rhizosphere  
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3 428 soil in both species (Fig. 5). This indicates the loss of newly-fixed C from roots was  
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5 429 similar in clipped and control treatments of both species.  
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9  
10 431 *Differences in the allocation of new C to NSC between clipped and control trees*

11  
12 432 Birch allocated more new C to SS than oak in all fractions and also significantly more  
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14 433 new C to starch, particularly in fast turnover fractions like leaves, young stems and fine-  
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16  
17 434 roots, while both species showed similar new C-allocation to starch in coarse roots and  
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19 435 main stems (Table 4, Fig. 6, Table S6 available as Supplementary Data at Tree  
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21 436 Physiology Online). In general, clipping had no significant effect on the allocation of  
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23 437 newly fixed C to storage (both SS and starch) during the next season after clipping  
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25  
26 438 (Table 4). However, in the case of the allocation to starch the response varied depending  
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28 439 on the species and the fraction and, consequently, the interaction terms between species  
29  
30 440 and treatment and the full interaction term were significant (Table 4). Simplified models  
31  
32 441 run separately per species and fraction indicated that, in birch, clipped trees allocated  
33  
34 442 significantly less C to starch in leaves and main stems (the latter marginally significant  
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36  
37 443 only) and marginally higher C to SS in current-year stems than control ones (Fig. 6).  
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39 444 Contrastingly, clipped oaks showed a trend for higher allocation of newly fixed C to  
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41 445 starch in current-year and main stems (Fig. 6), but results were only marginally  
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43  
44 446 significant.  
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## 48 448 **Discussion**

49  
50 449 In accordance with our first hypothesis, our results indicate that clipped birch and oak  
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52 450 trees shifted their C-allocation patterns above-ground as a means to recover from  
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55 451 defoliation. However, contrary to our expectations, such increased allocation to current-  
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58 452 year stems and leaves did not entail reductions in the allocation to the rhizosphere. As  
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3 453 initially expected, *B. pubescens* and *Q. petraea* showed differences in their vulnerability  
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5 454 and recovery strategies to clipping, the ring-porous species being less vulnerable than  
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7 455 the diffuse porous. These contrasting patterns could be explained by differences in their  
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9 456 C cycling after clipping. Defoliated oaks showed a more efficient recovery of their  
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11 457 canopy, which was supported by a larger allocation of new C into biomass, particularly  
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13 458 aboveground. However, this was associated with large decreases in the fine root  
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15 459 biomass of clipped oak trees. Although in both species clipped trees recovered NSC  
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17 460 pools faster than growth, the allocation of  $^{13}\text{C}$ -labelled photo-assimilates into starch and  
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19 461 SS was not increased as compared to controls. This indicates that, contrary to our  
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21 462 second hypothesis, no preferential allocation into NSC occurred during the first year  
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23 463 after clipping.  
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30 465 *Trees recovered from defoliation by increasing C allocation aboveground but*  
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32 466 *maintaining allocation to the rhizosphere*  
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35 467 Clipping led to a rapid reduction in tree growth (Palacio et al. 2011) and also in biomass  
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37 468 and NSC pools in current-year stems (the fraction directly affected by clipping  
38  
39 469 treatments). Over the course of the first year after clipping, trees managed to recover  
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41 470 initial differences in main stem diameter, total plant biomass and, in the case of oaks,  
42  
43 471 also tree height (Table 2). This was mainly a result of shifting allocation above-ground,  
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45 472 with increased allocation to current-year stems (almost double to that of control trees in  
46  
47 473 oak) and producing similar leaf biomass to controls (Fig. 2). In oak, these results were  
48  
49 474 consistent for both biomass allocation as a whole and when the proportion of new C  
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51 475 allocated to bulk biomass was considered (Fig. 4). The ability of trees to recover from  
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53 476 defoliation by increased above-ground allocation is a well-known phenomenon (Eyles  
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55 477 et al. 2009; Quentin et al. 2011). Such increased allocation may be achieved by a  
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3 478 combination of shifts in architecture, leaf morphology and C-allocation patterns (Eyles  
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5 479 et al. 2009).

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7 480 The removal of apical buds by browsing, clipping or defoliating insects  
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10 481 frequently leads to a decrease in apical dominance (due to changes in auxin fluxes,  
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12 482 Teichmann and Muhr 2015) with subsequent increases in lateral branch growth  
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14 483 (Haukioja et al. 1990; Wilson 1993). Clipped trees in our study showed a lower number  
15  
16 484 of terminal shoots, but increased branching was not detected in either of the two study  
17  
18 485 species. Clipped oaks showed similar lateral branch numbers to control trees, while  
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20 486 clipped birch trees had less lateral branches than controls. However, birch trees showed  
21  
22 487 a dramatic decrease in the number of short shoots produced after clipping (Table 2,  
23  
24 488 Palacio et al. 2011), which may be a direct consequence of the decreased apical  
25  
26 489 dominance after clipping (Haukioja et al. 1990). In addition, increases in the proportion  
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28 490 of leaves per bud (Millard et al. 2001) or in the foliage to wood ratio (Mizumachi et al.  
29  
30 491 2004) have been reported as mechanisms to increase above-ground allocation in clipped  
31  
32 492 trees. This was not the case in the trees included in this experiment, which showed  
33  
34 493 similar leaf biomass to control trees but increased current-year stem biomass. It seems,  
35  
36 494 therefore, that clipped trees in our experiment maximized the recovery of the canopy,  
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38 495 increasing investment into new stems while keeping a similar allocation to foliage. In  
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40 496 clipped birch trees, the decrease in short shoot number (likely in favour of long shoot  
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42 497 development), may be a mechanism to recover canopy spread and renewal of bud  
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44 498 numbers since, in this species, renewal buds are mostly borne in the long shoots  
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46 499 (MacDonald et al. 1984).

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53 500 Several previous studies have detected shifts in leaf morphology after defoliation  
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55 501 to compensate losses in the C-assimilating capacity of the canopy, frequently leading to  
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57 502 increased individual leaf area and weight and increased SLA (Fuenzalida et al 2019;  
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3 503 Millard et al. 2001; Piper and Fajardo 2015; Quentin et al. 2011; Trumble et al. 1993).  
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5 504 We did not detect any significant differences in leaf morphology between clipped and  
6  
7 505 control trees of either study species. Discrepancies with previous studies may be related  
8  
9 506 to differences in the type of disturbance applied and in the duration of experiments. For  
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11 507 example, Fuenzalida et al. (2019), Piper and Fajardo (2015) and Trumble et al. (1993)  
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13 508 evaluated responses after defoliating insects or treatments simulating defoliation by  
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15 509 arthropods, which may elicit a very different response by trees than clipping (Haukioja  
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17 510 et al. 1990). Further, Millard et al. (2001) and Quentin et al. (2011) applied clipping in  
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19 511 spring and measured the effects on leaf morphology at the end of the same growing  
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21 512 season, while in our study effects on leaf morphology were recorded at the end of the  
22  
23 513 next growing season after clipping, i.e. a much longer duration. Similarly, Eyles et al.  
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25 514 (2009) carried out a shorter duration experiment and did not detect any significant  
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27 515 effects on individual leaf area of *Eucalyptus globulus* five months after 40% defoliation.  
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33 516 Our results show that the increased above-ground allocation of clipped trees was  
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35 517 largely supported by currently-fixed (new) C, particularly in oak (Fig. 4). Increases in  
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37 518 photosynthetic rate have been repeatedly reported in defoliated trees (e.g. ~~Pinkard et al.~~  
38  
39 519 ~~2007~~; Pinkard et al. 1998; Vanderklein and Reich 1999). Although we did not measure  
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41 520 photosynthetic rates in our study, clipped oak trees showed higher new-C-allocation to  
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43 521 bulk biomass, compatible with increased C-fixing ability and with decreased respiratory  
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45 522 losses (see below). Both in control and clipped trees of both species, new C was  
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47 523 preferentially allocated to actively growing fractions, like leaves, young stems and fine  
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49 524 roots.  
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54 525 Although clipping induced increased C-allocation above-ground, our results  
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56 526 showed no significant effects of clipping on net deposition to soil in either of the two  
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58 527 species analyzed. Frost and Hunter (2008) obtained similar results in red oak (Frost and  
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3 528 Hunter 2008) and suggested that rhizodeposition might be a tightly controlled process  
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5 529 buffered against damage-induced shifts in C-allocation. Both species had a significant  
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7 530 effect on the  $\delta^{13}\text{C}$  isotopic composition of the soil, indicated by the depletion in  $^{13}\text{C}$   
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10 531 detected in rhizosphere vs. bulk soils. Such an effect was larger in birch than oak, likely  
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12 532 in relation to the larger C-fixing ability of the former. Consequently, trees were able to  
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14 533 impose changes in the C dynamics of soils, but such effects were not modified by  
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16  
17 534 clipping.

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21 536 *B. pubescens* trees were more severely affected than oaks by clipping.

22  
23 537 In agreement with the predictions by Foster (2017), the ring-porous species, *Q. petraea*,  
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25 538 was more efficient in recovering the biomass lost by clipping than the diffuse-porous, *B.*  
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27 539 *pubescens*. Clipped birch trees showed lower height and altered architecture (reduced  
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29 540 number of branches, terminal and lateral shoots) as compared to controls. Also, while  
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31 541 biomass losses in stems formed in 2007 (the cohort directly affected by clipping  
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33 542 treatments) were no longer significant at the end of the 2008 growing season in oaks,  
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35 543 they were still noticeable in birch. Foster (2017) hypothesized that ring-porous species  
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37 544 would be more resistant to spring defoliation than diffuse-porous ones owing to their  
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39 545 earlier wood phenology and increased C-stores (Barbaroux and Bréda 2002; Barbaroux  
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41 546 et al. 2003; Dougherty et al. 1979). We did not measure wood phenology in this study,  
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43 547 but it seems likely that this might have had an effect on the differential responses of  
44  
45 548 both species. In our experiment, clipping consisted of shoot removal in early July and  
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47 549 early September 2007, after the first and second flushes of shoot growth were finished.  
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49 550 If the differences in wood growth phenology between ring-porous and diffuse-porous  
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51 551 adult trees can be applied to saplings, oaks would be expected to have started wood  
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53 552 growth at least two weeks prior to the first defoliation event, while birch trees would be  
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3 553 just starting (Foster 2017). Foster (2017) further hypothesized that such differences in  
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5 554 damage due to wood growth phenology would entail a differential decrease in NSC  
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7 555 stores, which would be more severely decreased in diffuse-porous trees and further  
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9 556 exacerbated by their lower C-storage capacity. Our results do not confirm this  
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11  
12 557 prediction, since both species were equally able to recover NSC pools to the same level  
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14 558 as controls on the same year of clipping (except for the shoot cohort directly affected by  
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16 559 treatments). Similar fast recovery of NSC stores in defoliated trees has been previously  
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18 560 reported (Palacio et al. 2008; Piper et al. 2015; Puri et al. 2015).

21 561 Instead, our results show that the differential recovery ability of birch and oak  
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23 562 trees after clipping could be, at least partly, explained by the different effects of clipping  
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25 563 on their C-cycling, including differences in C-allocation. In accordance with previous  
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27 564 studies, oaks showed a larger reliance on storage than birch trees to support new growth  
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29 565 (Barbaroux et al. 2003; but see Palacio et al. 2011). This increased ability to re-mobilise  
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31 566 C-stores could have been crucial to support the re-growth of clipped oaks, at least  
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33 567 initially. Several previous studies have reported a positive relationship between NSC  
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35 568 storage and the re-growth ability of defoliated trees (Fierravanti et al. 2019; Kays and  
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37 569 Canham 1991; Luostarinen and Kauppi 2005). However, clipped oak trees invested  
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39 570 significantly less “old” C (and proportionally more “new” C) in their new growth than  
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41 571 control trees (Fig. 4). Consequently, while clipped oaks recovered to a larger extent than  
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43 572 clipped birch trees in our experiment, this was not linked to increased total NSC  
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45 573 remobilisation as measured at the end of the growing season.

51 574 The increased allocation of new C in clipped oaks to support re-growth as  
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53 575 compared to controls illustrates a shift in C cycling after damage resulting in  
54  
55 576 significantly more new C being allocated to most fractions in clipped vs. control oaks,  
56  
57 577 an effect not observed in birch. Such a change can be the result of increased C-fixation,  
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3 578 but also of decreased losses by respiration or rhizosphere allocation. Differences in C  
4  
5 579 allocation to the rhizosphere were not significant among treatments (see above).  
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7  
8 580 However, we cannot rule out the possibility that the increased allocation of new C  
9  
10 581 assimilates in oak was (at least partly) due to reduced respiratory losses, particularly in  
11  
12 582 relation to the drastic reduction in fine root biomass detected in clipped trees of this  
13  
14 583 species. Accordingly, the increased investment into above-ground components in oak  
15  
16 584 was associated with a reduced production of fine roots of 45% during the year after  
17  
18 585 clipping. These changes were not observed in birch, which maintained similar biomass  
19  
20 586 allocation below-ground between control and clipped trees. Increased fine root mortality  
21  
22 587 is a frequently reported process in defoliated trees (Frost and Hunter 2008; Tuomi et al.  
23  
24 588 1990; Vanderklein and Reich 1999; but see Endrulat et al. 2016; Kosola et al. 2001).  
25  
26 589 Tuomi et al. (1990) suggested that the reductions in fine root biomass after defoliation  
27  
28 590 could vary largely depending on the relative root biomass, the degree of reserve  
29  
30 591 depletion and the compensatory C gain of trees. All these three factors likely differed in  
31  
32 592 the two study species, which could explain their contrasting response. Oaks have  
33  
34 593 relatively high root:shoot ratios (Shaw 1974) as was the case also in this experiment  
35  
36 594 (Table 1). This means an increased non-productive biomass to support during re-growth  
37  
38 595 (Tuomi et al. 1990). The higher storage pool may not have been sufficient to recover  
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40 596 above-ground losses and maintain a large root biomass in oak (Tuomi et al. 1990).  
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49 598 *Clipping did not result in preferential C-allocation to storage in the long-term*

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51 599 Our study did not detect temporal differences in NSC concentrations in the different  
52  
53 600 organs of clipped and control trees throughout the first year of recovery, but NSC  
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55 601 concentrations of clipped trees of both species reached similar levels to those of control  
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57 602 trees by the end of the first growing season. This indicates that decreases in NSC stores  
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3 603 due to canopy re-growth, if any, were short-lived and fully compensated within less  
4  
5 604 than one year. Similar results have been previously reported in the literature (e.g.  
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7 605 Palacio et al. 2012; Puri et al. 2015; Wiley et al. 2013). In our experiment, the  
8  
9 606 replenishment of stores was likely supported by an increased C-fixing ability in clipped  
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11 607 trees, particularly in oak, as denoted by their higher new-C-allocation to bulk biomass.

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14 608 Despite NSC concentrations of clipped trees were rapidly restored to even higher  
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16 609 levels than control trees (Tables S2, S3), we did not detect a significant increase in new  
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18 610 C-allocation to storage in clipped trees in the first year after clipping. The only  
19  
20 611 significant effect of clipping on new C-allocation was a reduction in allocation to SS in  
21  
22 612 leaves of clipped birches. Wiley et al. (2017b; 2013) suggested that the growth of  
23  
24 613 defoliated trees would be largely limited by C-availability, first by the decrease in leaf  
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26 614 area directly related to defoliation, and then by a prioritized allocation to storage over  
27  
28 615 growth to secure tree survival under future potential defoliation events. They argued  
29  
30 616 that the fact that NSC stores were replenished to control levels did not necessarily mean  
31  
32 617 tree growth was not limited by C-availability, since prioritized allocation to storage over  
33  
34 618 growth could still proceed (Wiley and Helliker 2012). Two recent experiments have  
35  
36 619 experimentally demonstrated that NSC concentrations can be maintained to control  
37  
38 620 levels over periods of C-limitation by preferential allocation of C into storage, calling  
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40 621 for a cautious use of NSC concentrations to predict the C-status of trees (Weber et al.  
41  
42 622 2019; 2018). In both experiments, trees subjected to low or no illumination were  
43  
44 623 progressively C-deprived, reaching minimum SS and starch ~~concentrations as low as~~  
45  
46 624 ~~2% and 1%, respectively. Below these minimum~~ thresholds, ~~below which~~ tree survival  
47  
48 625 was impaired. In both cases, re-illumination resulted in a period of reduced growth and  
49  
50 626 refilling of NSC stores ~~up to a certain threshold. Weber et al. (2019) reported a~~  
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52 627 ~~threshold of 25% and 30% replenishment of NSC concentrations before the growth rate~~  
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3 | 628 ~~increased in *Acer* and *Quercus* saplings, respectively.~~ These results indicate that  
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5 | 629 prioritized allocation to storage over growth does occur in C-starved trees (with very  
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7 | 630 low NSC levels), and that such prioritization is arrested once a certain level of recovery  
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9 | 631 of NSC is achieved.

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11  
12 | 632 In contrast to the experiments in Weber et al. (2019; 2018), our trees were not C-  
13  
14 | 633 starved. We did not find the very significant depletion of NSC reported by these  
15  
16 | 634 previous studies (Weber et al. 2019; 2018). Consequently, our trees probably did not  
17  
18 | 635 prioritize allocation to NSC over growth. Starch and SS concentrations of defoliated  
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20 | 636 trees are normally not depleted below the C-starvation thresholds detected by Weber et  
21  
22 | 637 al. (2019; 2018), even after severe treatments (e.g. Kays and Canham 1991; Palacio et  
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24 | 638 al. 2012; ~~Raitio et al. 1994~~; Vanderklein and Reich 1999, but see Kosola et al. 2001;  
25  
26 | 639 Puri et al. 2015). This seems to indicate C-limitation after defoliation in trees is  
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28 | 640 normally short-lived and of low magnitude.  
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## 34 | 642 **Conclusions**

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37 | 643 Our results show that clipping triggers a shift in biomass allocation aboveground  
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39 | 644 favouring the recovery of the canopy both in oak and birch trees. However, such a shift  
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41 | 645 does not entail a decrease in C-allocation to the rhizosphere, which seems to be a tightly  
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43 | 646 regulated process. Future research on the mechanisms behind such tight regulation  
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45 | 647 would greatly contribute to our understanding on the effects of defoliation on tree C-  
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47 | 648 cycling and its impact on belowground processes. The observed differences in the  
48  
49 | 649 recovery strategies of the two study species could have potential implications for their  
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51 | 650 vulnerability under different browsing frequencies. The ring porous species, *Q. petraea*,  
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53 | 651 showed a faster recovery of its canopy after clipping than the diffuse porous, *B.*  
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55 | 652 *pubescens*. However, this came at the cost of a marked decrease in the fine root biomass  
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3 653 of oak, which raises questions on the potential consequences for the nutrition of the tree  
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5 654 and its vulnerability to sustained browsing over longer time periods. Finally, despite the  
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7 655 different effect of clipping on the C-allocation of study species, none of them increased  
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9 656 new C allocation to storage one year after damage. This indicates that clipping does not  
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11 657 entail a sustained preventative allocation of C into storage in the long term.  
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### 16 17 659 **Data and Materials Availability**

18  
19 660 Authors agree to make experimental data and materials available to third party academic  
20  
21 661 researchers upon reasonable request.  
22  
23  
24 662

### 25 26 663 **Supplementary Data**

27  
28 664 The following supporting documents are provided as additional content:

29  
30 665 - Table S1. Results of glms on the effect of clipping on the biomass (BM), and soluble  
31  
32 666 sugars (SS) and starch pools and concentrations of the different fractions of *B.*

33  
34 667 *pubescens* and *Q. petraea* trees harvested at the beginning of the  $\delta^{13}\text{C}$ -labelling  
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36 668 experiment in April 2008.

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38 669 - Table S2. Soluble sugars (SS) and starch concentrations ( $\text{mg g}^{-1}$ ) in the different plant  
39  
40 670 fractions of *B. pubescens* and *Q. petraea* trees harvested at the beginning of the  $\delta^{13}\text{C}$ -

41  
42 671 labelling experiment in April 2008.

43  
44 672 - Table S3. Soluble sugars (SS) and starch pools (as % of the total SS or starch pool in  
45  
46 673 the plant) in the different plant fractions in *B. pubescens* and *Q. petraea* trees harvested

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48 674 at the beginning of the  $\delta^{13}\text{C}$ -labelling experiment in April 2008.

49  
50 675 - Table S4. Isotopic composition ( $\delta^{13}\text{C}$ ; ‰) of the different fractions of *B. pubescens*  
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52 676 and *Q. petraea* saplings harvested in August 2008.  
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3 677 - Table S5. Results of glms on the effect of “Species” and “Treatment” on the allocation  
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5 678 of new C to bulk biomass of the different fractions of *B. pubescens* and *Q. petraea* trees  
6  
7 679 harvested in August 2008.

8  
9  
10 680 - Table S6. Results of glms on the effect of “Species” and “Treatment” on the allocation  
11  
12 681 of new C to soluble sugars (SS) and starch in the different fractions of *B. pubescens* and  
13  
14 682 *Q. petraea* trees harvested in August 2008.

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19 684 **Conflict of interest**

20  
21 685 None declared.

22  
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25  
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33  
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39  
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43  
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45  
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51 698 **Authors' Contributions**

52  
53 699 SP, PM, AH and EP designed the study; SP, EP and PM implemented continuous  $\delta^{13}\text{C}$ -  
54  
55 700 labelling. SP, EP, PM, MM, GL, SN and AAR run carbohydrate and stable isotope

1  
2  
3 701 analyses. SP, PM and SN analysed data. All authors interpreted results. SP wrote the  
4  
5 702 manuscript receiving revisions from all authors.  
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3 954 **Figure legends**  
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5 955 **Fig. 1.** Experimental design with the indication of the main treatments (clipping – red  
6 arrows-,  $^{13}\text{C}$ -labelling), growth measurements (blue arrows) and harvests (black  
7 956 arrows) applied to *B. pubescens* and *Q. petraea* saplings.  
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12 958 **Fig. 2.** Biomass allocation to different tree fractions in clipped and control trees of *B.*  
13 *pubescens* and *Q. petraea* harvested in August 2008 (ca. one year after clipping).  
14 959 Average values are shown for each fraction. L = Leaves, 0-St = current year stems, 1-St  
15 = one-year-old stems, 2-St = two-year-old stems, MS = main stems, CR = coarse roots,  
16 960 FR = fine roots. Significant differences between treatments within species are indicated  
17 961 by asterisks: \* =  $P < 0.10$ , \*\* =  $P < 0.05$ .  
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26 964 **Fig. 3.** Differences between clipped (grey bars) and control (black bars) *B. pubescens*  
27 and *Q. petraea* saplings in the allocation of SS and starch pools to different plant  
28 965 fractions one year after clipping (trees harvested in August 2008). Asterisks denote  
29 966 significant differences between treatments within a given species at  $\alpha = 0.10$  (\*) and  $\alpha =$   
30 967 0.05 (\*\*).  
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38 969 **Fig. 4.** Differences between clipped (grey bars) and control (black bars) *B. pubescens*  
39 and *Q. petraea* saplings in the allocation of newly fixed C to the different plant organs  
40 970 one year after clipping (trees harvested in August 2008). Asterisks denote significant  
41 971 differences between treatments within a given species at  $\alpha = 0.05$ .  
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46 973 **Fig 5.** Isotopic composition ( $\delta^{13}\text{C}$ ; ‰) of the rhizosphere and bulk soil collected  
47 underneath control (white dots) and clipped (black dots) *B. pubescens* and *Q. petraea*  
48 974 trees harvested in August 2008 (ca. one year after clipping). No significant differences  
49 975 between treatments were detected at  $\alpha = 0.05$ .  
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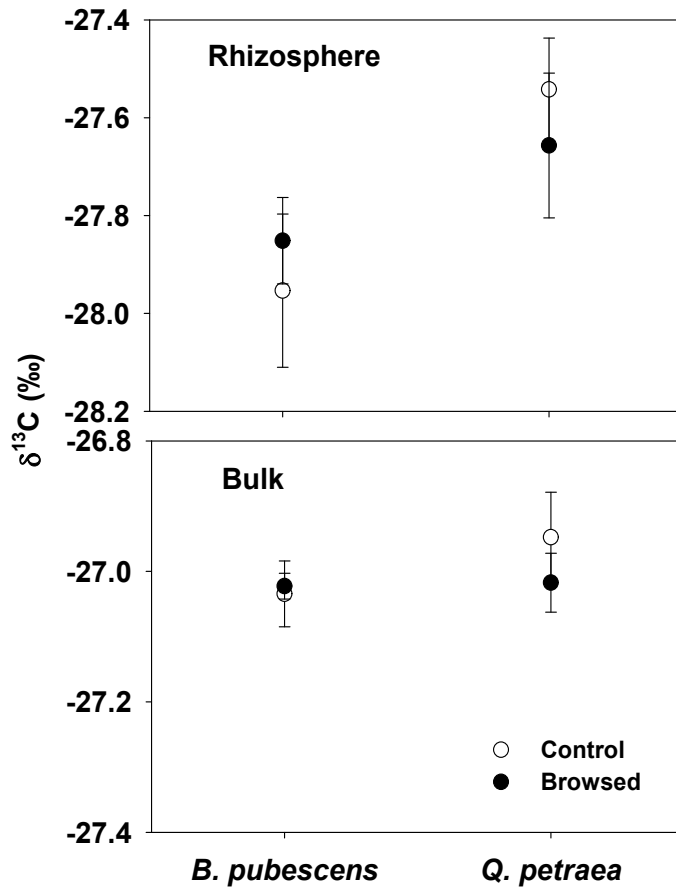
56 977 **Fig 6.** Differences between control (black bars) and clipped (grey bars) *B. pubescens*  
57 and *Q. petraea* saplings in the allocation of newly fixed C to SS and starch to different  
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3 979 plant fractions on the year after clipping (trees harvested in August 2008). Asterisks  
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5 980 denote significant differences between treatments within a given species at  $\alpha = 0.10$  (\*),  
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8 981 and  $\alpha = 0.05$  (\*\*).  
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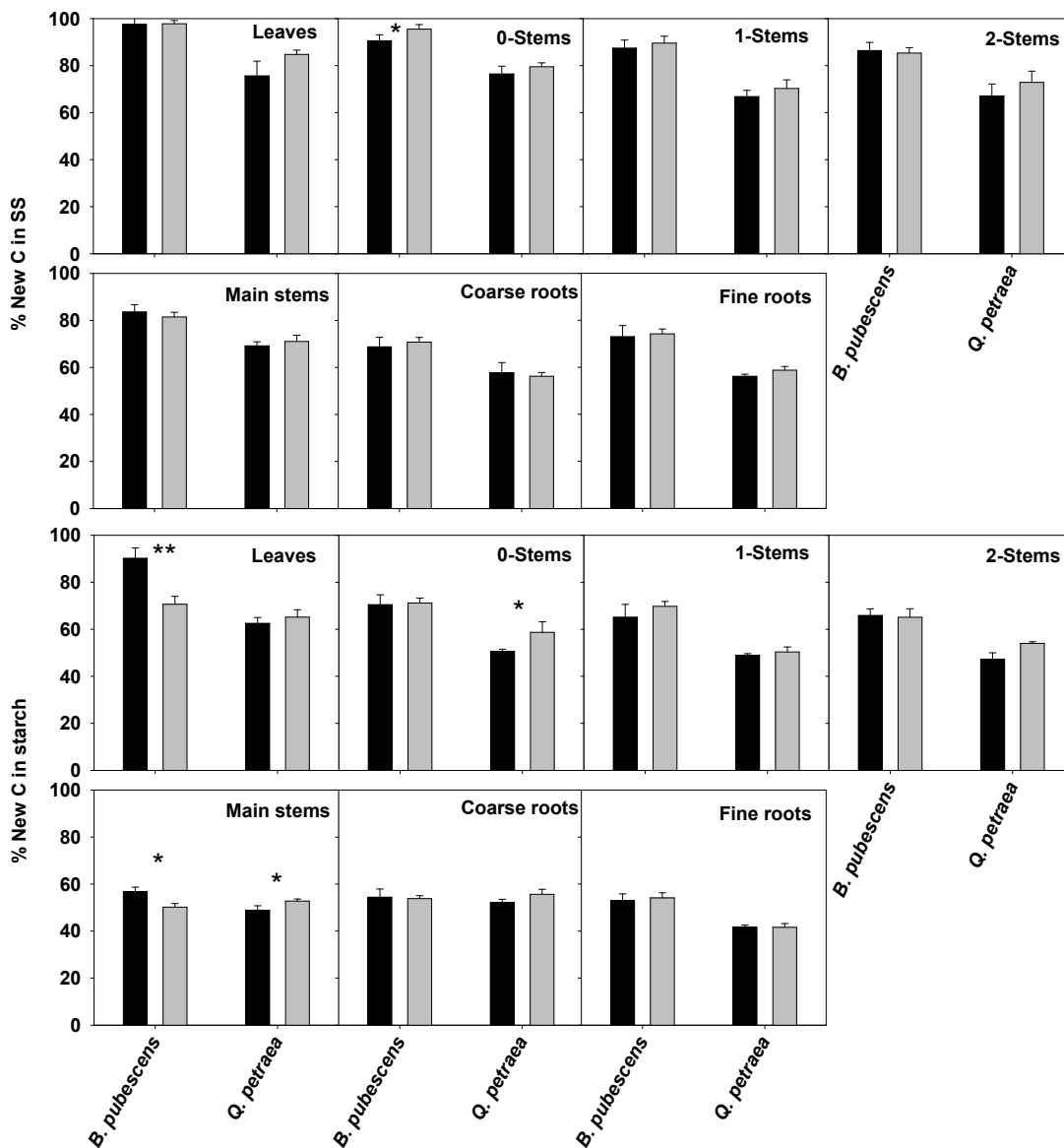
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3 **1 No preferential C-allocation to storage over growth in clipped birch and oak**  
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5 **2 saplings**  
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11 **4 Sara Palacio<sup>1,2\*</sup>, Eric Paterson<sup>2</sup>, Alison J. Hester<sup>2</sup>, Salvador Nogués<sup>3</sup>, Gladys Lino<sup>3,</sup>**  
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13 **4, Alba Anadon-Rosell<sup>3, 5</sup>, Melchor Maestro<sup>6</sup> and Peter Millard<sup>7</sup>**  
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3 **23 Abstract**  
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6 **24** Herbivory is one of the most globally distributed disturbances affecting C-cycling in  
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8 **25** trees, yet our understanding of how it alters tree C-allocation to different functions like  
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10 **26** storage, growth or rhizodeposition is still limited. Prioritized C-allocation to storage  
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12 **27** replenishment vs. growth could explain the fast recovery of C-storage pools frequently  
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14 **28** observed in growth-reduced defoliated trees. We performed continuous <sup>13</sup>C-labelling  
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16 **29** coupled to clipping to quantify the effects of simulated browsing on the growth, leaf  
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18 **30** morphology and relative allocation of stored vs. recently assimilated C to the growth  
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20 **31** (bulk biomass) and non-structural carbohydrate (NSC) stores (soluble sugars and starch)  
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22 **32** of the different organs of two tree species: diffuse-porous (*Betula pubescens*) and ring-  
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24 **33** porous (*Quercus petraea*). C-transfers from plants to bulk and rhizosphere soil were  
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26 **34** also evaluated. Clipped birch and oak trees shifted their C-allocation patterns above-  
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28 **35** ground as a means to recover from defoliation. However, such increased allocation to  
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30 **36** current-year stems and leaves did not entail reductions in the allocation to the  
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32 **37** rhizosphere, which remained unchanged between clipped and control trees of both  
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34 **38** species. *B. pubescens* and *Q. petraea* showed differences in their vulnerability and  
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36 **39** recovery strategies to clipping, the ring-porous species being less affected in terms of  
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38 **40** growth and architecture by clipping than the diffuse-porous. These contrasting patterns  
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40 **41** could be partly explained by differences in their C cycling after clipping. Defoliated  
41  
42 **42** oaks showed a faster recovery of their canopy biomass, which was supported by  
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44 **43** increased allocation of new C, but associated with large decreases in their fine root  
45  
46 **44** biomass. Following clipping, both species recovered NSC pools to a larger extent than  
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48 **45** growth, but the allocation of <sup>13</sup>C-labelled photo-assimilates into storage compounds was  
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50 **46** not increased as compared to controls. Despite their different response to clipping, our  
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3 47 results indicate no preventative allocation into storage occurred during the first year  
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5 48 after clipping in either of the species.  
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11 50 **Keywords:** *Quercus petraea*, *Betula pubescens*, Carbon (C) allocation,  $\delta^{13}\text{C}$  stable  
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13 51 isotopes, non-structural carbohydrates, C-storage, below-ground allocation.  
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## 53 **Introduction**

54 Trees account for ca. 90% of the global biomass of carbon (C) (Körner 2003), and  
55 hence play a fundamental role in global C dynamics. C-allocation patterns in trees may  
56 shift depending on multiple factors, including age (Hartmann et al. 2018), phenology  
57 (Palacio et al. 2018), environmental conditions like water and light availability or  
58 temperature (Domisch et al. 2001; Messier and Nikinmaa 2000; Weber et al. 2019) and  
59 disturbances (Canham et al. 1994; Van der Heyden and Stock 1995). C-allocation  
60 within the tree biomass may determine tree vulnerability to environmental stress and  
61 disturbance (Canham et al. 1999; Wiley and Helliker 2012). Further, C-allocation to  
62 different functions like storage, growth, reproduction or rhizodeposition may affect the  
63 amount of C cycled and sequestered by trees (Hartmann et al. 2018). Understanding the  
64 response of tree C-allocation patterns to different factors may be crucial to predict the  
65 response of trees to global change (Körner 2003; Wiley and Helliker 2012).

66 Herbivory is one of the most globally distributed disturbances affecting C-  
67 cycling patterns in trees (Clark et al. 2010). Defoliation by herbivores reduces canopy  
68 leaf area causing a decrease in the net C gain of trees by current photosynthesis and  
69 altering the balance between C sinks and sources (Trumble et al. 1993). This may lead  
70 to important changes in C-allocation patterns, which can influence the environment by  
71 changes in below-ground C inputs (Eyles et al. 2009; Pinkard and Beadle 1998).  
72 Depending on the severity of damage, C demands of growing sinks may be supplied  
73 temporarily from storage (Pinkard et al. 1998; Quentin et al. 2011; Van der Heyden and  
74 Stock 1995), namely non-structural carbohydrates (NSC) and lipids, some of which can  
75 be mobilised to support growth or other plant functions (Chapin et al. 1990).  
76 Accordingly, several studies have reported a decrease in starch pools after defoliation in  
77 deciduous (Canham et al. 1994; Kosola et al. 2001; Van der Heyden and Stock 1995)

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3 78 and evergreen tree species (Ericsson et al. 1985; Fierravanti et al. 2019). However, trees  
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5 79 are able to compensate to some degree for loss of foliage by changing allocation  
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8 80 patterns (e.g. favouring foliage production), upregulating photosynthesis and changing  
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10 81 leaf morphology (Fuenzalida et al. 2019; Pinkard and Beadle 1998). Recovery from  
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12 82 light defoliation is considered to rely mainly on current photo-assimilates produced by  
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14 83 surviving foliage (Barry et al. 2011) and does not normally cause a significant decrease  
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17 84 in NSC pools (Tschaplinski and Blake 1994; Van der Heyden and Stock 1995). In the  
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19 85 case of moderate or severe defoliation, decreases in NSC concentrations tend to be  
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21 86 transient and of short duration, becoming non-significant over the course of a growing  
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23 87 season, while the effects on tree growth seem to be more long-lasting (Palacio et al.  
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26 88 2008; Piper et al. 2015; Puri et al. 2015). Sometimes defoliated trees show even higher  
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28 89 NSC concentrations than undefoliated controls (Palacio et al. 2012; Piper et al. 2015).

30       The differential dynamics of growth vs. recovery of NSC pools in defoliated  
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32 90 trees have been interpreted in relation to two, non-exclusive processes: 1) a C-sink  
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34 91 limitation to growth due to reductions in the numbers of buds, limiting levels of non-C  
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36 92 reserves, hormonal changes or allometric adjustments in response to reduced leaf area,  
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38 93 leading to surplus-C being allocated to storage (Palacio et al. 2012; Palacio et al. 2008;  
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40 94 Piper et al. 2015; Puri et al. 2015; Schmid et al. 2017); 2) a preventative prioritized C-  
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42 95 allocation to storage over growth, ultimately leading to C-limitation (Piper et al. 2015;  
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44 96 Puri et al. 2015; Wiley et al. 2017a; Wiley and Helliker 2012; Wiley et al. 2013).  
45  
46 97 Preferential allocation of C to NSC over growth has recently been demonstrated in C-  
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48 98 starved plants subjected to prolonged shading (Weber et al. 2019) or complete darkness  
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50 99 (Weber et al. 2018). Nevertheless, its occurrence in other potentially C-limiting  
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52 100 conditions such as defoliation remains equivocal (Wiley et al. 2017a; 2013).  
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3 102 In addition to changes in C-allocation among tree organs, defoliation can induce  
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5 103 shifts in C-transfers below-ground, influencing soil microbial communities (Bardgett  
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7 104 and Wardle 2003 but see Barto and Rillig 2010) and nutrient cycling (Ayres et al.  
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10 105 2004). Approximately 50% of the C produced by woody plants is allocated below-  
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12 106 ground, either directly to the roots, or as rhizodeposition of C exudates from roots to the  
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14 107 surrounding soil (Giardina et al. 2005). Defoliation can reduce below-ground C-  
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16 108 allocation by enhancing fine root mortality, particularly in trees (Bryant et al. 1993;  
17  
18 109 Tuomi et al. 1990; Vanderklein and Reich 1999, but see Endrulat et al. 2016; Kosola et  
19  
20 110 al. 2001). In contrast, in herbaceous plants herbivory can increase short-term allocation  
21  
22 111 of C below-ground (Orians et al. 2011), as has also been found for *Populus* spp. (Babst  
23  
24 112 et al. 2005). Defoliation has been demonstrated to elicit short-term increases in the flux  
25  
26 113 of C to root exudates in grasses (Paterson et al. 2005), while the detection of effects in  
27  
28 114 woody species remains elusive (Ayres et al. 2004; Frost and Hunter 2008). In general,  
29  
30 115 there is a lack of information related to below-ground responses of woody plants to  
31  
32 116 defoliation.

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37 117 Differences in wood anatomy have also been assumed to entail differences in C-  
38  
39 118 allocation dynamics (Barbaroux and Bréda 2002), with putative consequences on the  
40  
41 119 response of trees to defoliation (Foster 2017). Ring-porous species complete part of  
42  
43 120 earlywood growth (including large earlywood vessel formation) before bud burst in  
44  
45 121 spring (Dougherty et al. 1979). This phenology is putatively a result of winter embolism  
46  
47 122 of large-diameter vessels, and the need to produce a new set of xylem vessels prior to  
48  
49 123 bud burst to supply newly emerging leaves with water (Lechowicz 1984). Contrastingly,  
50  
51 124 diffuse-porous species have only small xylem vessels and winter embolism has  
52  
53 125 relatively less impact on the hydraulic conductivity of the tree, so leaf expansion can  
54  
55 126 proceed using xylem formed in the previous growing season, without the need to  
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1  
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3 127 produce new radial stem growth (Lechowicz 1984). The consequence for C-cycling is  
4  
5 128 that ring-porous species show greater seasonal variations in NSC pools and  
6  
7 129 concentrations, and a relatively greater dependence on the remobilization of stored NSC  
8  
9 130 for earlywood growth in spring, than diffuse-porous species (Barbaroux and Bréda  
10  
11 131 2002; Barbaroux et al. 2003, but see Palacio et al. 2011). It has recently been suggested  
12  
13 132 that these differences in C storage and allocation underline potential differences  
14  
15 133 between ring-porous and diffuse-porous species in the vulnerability to spring  
16  
17 134 defoliation, the former being more resistant to defoliation owing to their larger C-stores  
18  
19 135 and advanced wood growth phenology (Foster 2017). Nevertheless, to our knowledge  
20  
21 136 this possibility has never been explored experimentally.

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26 137 We performed continuous  $^{13}\text{C}$ -labelling coupled to a clipping experiment to  
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28 138 quantify the effects of simulated browsing on the relative allocation of stored and  
29  
30 139 recently assimilated C to growth (bulk biomass) and NSC (soluble sugars and starch) of  
31  
32 140 the different organs of two tree species with contrasting wood anatomy: diffuse-porous  
33  
34 141 downy birch (*Betula pubescens* Ehrh.) and ring-porous sessile oak (*Quercus petraea*  
35  
36 142 [Matt.] Liebl.). Transfers from the plants to bulk (i.e. not in direct contact with tree  
37  
38 143 roots) and rhizosphere soil were also evaluated. Clipping was selected to mimic the  
39  
40 144 effects of browsing: a major factor hampering the regeneration of native forests  
41  
42 145 worldwide (Gill 2006; Hester et al. 2004). The use of continuous  $^{13}\text{C}$ -labelling at close-  
43  
44 146 to-ambient concentrations was chosen as a quantitative mean to separate current from  
45  
46 147 stored C-assimilates, estimate C-allocation to different organs and C-compounds over  
47  
48 148 the course of the growing season and track allocation below-ground, without the  
49  
50 149 potential drawbacks of pulse-chase labelling (see Paterson et al. 2009). We  
51  
52 150 hypothesized that clipping would lead to: (i) increased C-allocation above-ground vs.  
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54 151 below-ground (i.e. roots and the rhizosphere); (ii) increased allocation of new C into  
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3 152 storage; and (iii) reduced growth and C storage, more noticeable in birch than in oak,  
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5 153 owing to the ring-porous wood anatomy and subsequent larger storage C-pools of the  
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8 154 latter (Foster 2017).  
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## 11 156 **Materials and methods**

### 12 157 *Experimental set up*

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15 158 The experimental set up was the same as described in Palacio et al. (2011). In brief, in  
16  
17 159 2007 we applied two clipping treatments: control (unclipped) and clipped (i.e. 66%  
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19 160 shoots removal in two consecutive dates: July and September 2007) to two-year-old  
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21  
22 161 sessile oak (*Quercus petraea* [Matt.] Liebl.) and downy birch (*Betula pubescens* Ehrh.)  
23  
24 162 saplings planted in pots (Fig. 1). In April 2008, before bud burst, five trees of each  
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26 163 species and treatment were harvested to account for differences in biomass and NSC  
27  
28 164 allocation in the short-term. At that same time, five extra trees of each species and  
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30 165 treatment were moved into a polytunnel with altered  $\delta^{13}\text{C}$  air composition to take part in  
31  
32 166 a continuous  $\delta^{13}\text{C}$ -labelling experiment, while five control trees of each species were  
33  
34 167 left at the greenhouse to serve as “ambient” trees. The aim of the continuous labelling  
35  
36 168 was to separate newly fixed C from “old” C. In August 2008, trees from the C-labelling  
37  
38 169 experiment were harvested to evaluate differences in new C allocation to bulk biomass  
39  
40 170 and NSC (SS and starch) between clipped and control trees of both species one year  
41  
42 171 after clipping (Fig. 1). “Ambient” trees were harvested in November 2008 to provide  
43  
44 172 natural abundance  $\delta^{13}\text{C}$  values of the different organs of both species to be used in  
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46 173 calculations. Further details of these experimental procedures follow.  
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### 55 175 *Clipping experiment*

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3 176 Trees of both study species were lifted from a nursery while dormant (5 April 2007) and  
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5 177 planted in 44 l pots filled with gravel at the bottom for drainage and freely-drained soil  
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7 178 derived from granite and granitic gneiss (Countesswells Association, Glentworth and  
8  
9 179 Muir 1963). At planting, saplings were approximately 0.3-0.5 m high, a stage  
10  
11 180 considered highly vulnerable to large herbivore browsing in nature (Gill 2006; Hester et  
12  
13 181 al. 2000; Hester et al. 1996). After planting, saplings were moved into an unheated  
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15 182 greenhouse and 20 trees of each species were randomly allocated into “clipped” and  
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17 183 “control” treatments, leading to 10 replicates per species and treatment combination.  
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19 184 Five extra trees per species were allocated to the “ambient” group, which did not  
20  
21 185 receive clipping or  $^{13}\text{C}$ -labelling. Trees were numbered and positioned in the  
22  
23 186 greenhouse following a Latin square design. Between April and November 2007, soil  
24  
25 187 was kept moist with tap water without exceeding field capacity and saplings received  
26  
27 188 0.5 l of a nutrient solution with  $3.0 \text{ mol N m}^{-3}$  as  $\text{NH}_4\text{NO}_3$ ,  $1.33 \text{ mol m}^{-3} \text{ Na}_2\text{HPO}_4 \cdot 12$   
28  
29 189  $\text{H}_2\text{O}$  and  $1 \text{ mol m}^{-3} \text{ K}_2\text{SO}_4$  once per week, to remove any potential nutrient limitation to  
30  
31 190 growth. A natural photoperiod was used and the greenhouse ventilated to provide  
32  
33 191 temperatures close to ambient. To account for initial tree variability and avoid potential  
34  
35 192 confounding effects on tree growth, morphological measurements (tree height, length,  
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37 193 stem diameter, and number of short shoots and long shoots) were taken from every tree  
38  
39 194 at planting and prior to each clipping and harvest.

46  
47 195 Clipping was applied as in Palacio et al. (2011) by removing 66% of current-  
48  
49 196 year shoots (2 out of every 3 current-year shoots) in early July and early September  
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51 197 2007, after the first and second flushes of shoot growth were finished (Fig. 1). This  
52  
53 198 intensity of damage was selected to reproduce high densities of browsing animals  
54  
55 199 (Speed et al. 2011). In birch, clipping treatments were designed to reproduce browsing  
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57 200 damage by red deer or sheep by removing current-year long-shoots (including stems,  
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3 201 buds and leaves) up to the maximum stem diameters normally eaten by red deer or  
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5 202 sheep (Shipley et al. 1999). In oaks, clipped shoots were selected to ensure a decrease in  
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7 203 total tree leaf area of approx. 66% owing to the highly variable shoot length and fewer  
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9 204 shoots of this species. While the use of clipping to simulate browsing has received some  
10  
11 205 criticism (Baldwin 1990), woody plant responses to well-simulated damage do not  
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13 206 differ significantly from responses to real herbivore damage (Bergman 2002; Hester et  
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15 207 al. 2004).

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21 209 *Short-term effects of clipping on biomass and carbohydrate allocation*

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23 210 Twenty dormant saplings (five of each species and clipping treatment) were removed  
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25 211 from their pots on 2 April 2008 and separated into: one and two-year-old stems (i.e.  
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27 212 stems formed in 2007 and 2006, respectively), woody stems (>2 years), coarse roots (>  
28  
29 213 2 mm diameter) and fine roots (< 2mm diameter). Samples were freeze-dried and  
30  
31 214 weighed (*ca.* 0.005 mg) and then milled to a fine powder in a ball mill (Retsch Mixer  
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33 215 MM301, Leeds, UK).

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37 216 Soluble sugars (SS) were extracted with 80% (v/v) ethanol and their  
38  
39 217 concentration determined using the phenol-sulphuric method as modified by Buysse and  
40  
41 218 Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after  
42  
43 219 ethanol extractions were reduced (i.e. enzymatically) to glucose and analyzed as  
44  
45 220 described in Palacio et al. (2007).

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51 222 *Continuous <sup>13</sup>C labelling experiment*

52  
53 223 On 1 April 2008, five saplings from each species and clipping treatment combination  
54  
55 224 were transferred to an aluminium and polythene tunnel, 2.4 m wide, 3.0 m long and 2.2  
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57 225 high (Super 8 Hobby Tunnel, Northernpolytunnels, Colne, UK) as described previously  
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3 226 (Palacio et al., 2011; Fig. 1). At this time, trees were only just starting to break bud. The  
4  
5 227 polytunnel was supplied with air having CO<sub>2</sub> with a depleted <sup>13</sup>C-signature (relative to  
6  
7 228 atmospheric CO<sub>2</sub>), in order to differentiate current (new) from previous (old) plant  
8  
9 229 assimilates (Nogués et al. 2004; Paterson et al. 2009). This was achieved by partially  
10  
11 230 scrubbing CO<sub>2</sub> from the air using a CO<sub>2</sub>-scrubber unit (Texol, Dundee, UK) that  
12  
13  
14 231 reduced the CO<sub>2</sub> concentration to 74-103 μmol mol<sup>-1</sup>. The scrubbed air was then mixed  
15  
16 232 with CO<sub>2</sub> from a gas cylinder (BOC, Worsley, UK) with a δ<sup>13</sup>C-signature of -34.0 ‰,  
17  
18 233 using Brooks 580s thermal mass flow controllers, interfaced with a Brooks control unit  
19  
20 234 (both Flotech Solutions Ltd, Stockport, UK). Resulting CO<sub>2</sub> concentrations inside the  
21  
22 235 polytunnel averaged 332 ppm and had an average δ<sup>13</sup>C of -21.4‰. Temperature inside  
23  
24 236 the polytunnel was checked regularly with a shielded thermometer. On the 6<sup>th</sup> of May  
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26 237 2008 we installed a shade mesh intercepting ~30% of the light on top of the polytunnel  
27  
28 238 to reduce warming.

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33 239 Trees were distributed within the polytunnel following a Latin square design,  
34  
35 240 which was changed in the middle of the experiment. They regularly received 0.5 l of the  
36  
37 241 same nutrient solution described above. We took initial and final tree morphological  
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39 242 measurements (as described above) at the beginning and at the end of the <sup>13</sup>C-labelling  
40  
41 243 period. On 5th August 2008, four months after the beginning of the <sup>13</sup>C-labelling, trees  
42  
43 244 inside the polytunnel were harvested for analysis (Fig. 1). At this time, leaf senescence  
44  
45 245 was starting and most of the annual growth had been completed. Harvested trees were  
46  
47 246 separated into: current-year (formed in 2008), one and two-year-old stems (formed in  
48  
49 247 2007 and 2006, respectively), woody stems (>2 years), coarse roots (> 2mm diameter)  
50  
51 248 and fine roots (< 2mm diameter). A three-year-old branch was clipped off each tree to  
52  
53 249 measure leaf area, individual leaf weight and specific leaf area according to the  
54  
55 250 protocols in Cornelissen et al. (2003). Rhizosphere and bulk soil were harvested from  
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1  
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3 251 each pot. Rhizosphere soil was collected by separating roots from the soil, gently  
4  
5 252 shaking them and then submerging fine roots in distilled water. Samples were freeze-  
6  
7 253 dried and weighed (ca. 0.005 mg). Samples were milled to a fine powder in a ball mill  
8  
9 254 (Retsch Mixer MM301, Leeds, UK) and analysed for NSC as described above.  
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#### 15 256 *Ambient trees*

16  
17 257 In early November 2008, the five non-clipped, "ambient", saplings of each species were  
18  
19 258 harvested from the greenhouse for analysis of the  $\delta^{13}\text{C}$  at natural abundance in the bulk  
20  
21 259 biomass of the same fractions considered for  $^{13}\text{C}$ -labelled trees. Throughout 2008,  
22  
23 260 growth conditions for these trees were similar to those in 2007 (see above). At the time  
24  
25 261 of harvest, trees were shedding their leaves and radial growth had been completed.  
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#### 29 263 *C isotope analysis*

30  
31 264 The  $^{13}\text{C}$  signature of samples was measured by continuous flow isotope ratio mass  
32  
33 265 spectrometer (Thermo Finnigan Delta Plus<sup>advantage</sup>) interfaced to an elemental analyser  
34  
35 266 (Thermo FlashEA1112, Thermo Finnigan, Bremen, Germany). Data were expressed as  
36  
37 267  $\delta^{13}\text{C}_{\text{V-PDB}}$ :

$$38 \quad \delta^{13}\text{C} (\text{‰}) = (R_S / R_{\text{VPDB}} - 1) \times 1000$$

39  
40 269 where  $R_S$  and  $R_{\text{VPDB}}$  are the molecular abundance ratios of carbon isotopes ( $^{13}\text{C}/^{12}\text{C}$ ), of  
41  
42 270 the sample and international standard (Vienna Pee Dee Belemnite), respectively. Long-  
43  
44 271 term precision for quality control standards (milled flour) was  $\delta^{13}\text{C}_{\text{V-PDB}}$ :  $-26.0 \pm$   
45  
46 272  $0.24\text{‰}$  (mean  $\pm$  SD,  $n=187$ ).  $\delta^{13}\text{C}$  was measured in bulk plant biomass, bulk soil and  
47  
48 273 rhizosphere soil. Measurements of  $\delta^{13}\text{C}$  in SS and starch followed compound-specific  
49  
50 274 analyses as detailed below.  
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275  $\delta^{13}\text{C}$  in NSC were measured following the procedure in Tcherkez et al. (2003).  
 276 In brief, leaf powder was suspended with 1 mL of distilled water in an Eppendorf tube  
 277 (Eppendorf Scientific, Hamburg, Germany). After centrifugation, starch was extracted  
 278 from the pellet by HCl solubilization. Soluble proteins of the supernatant were heat  
 279 denatured and precipitated and SS and organic acids of the protein-free extract were  
 280 separated by HPLC. After lyophilization, 200 mg of purified starch were weighted into  
 281 tin capsules (Courtage Analyze Service, Mont Saint-Aignan, France) for isotope  
 282 analysis. Determinations of  $\delta^{13}\text{C}$  in NSC were conducted at the *Centres Científics i*  
 283 *Tecnològics* (CCiT) of the University of Barcelona using an elemental analyser  
 284 (EA1108, Series 1, Carbo Erba Instrumentazione, Milan, Italy) coupled to an isotope  
 285 ratio mass spectrometer (Delta C, Finnigan, Mat., Bremen, Germany) operating in  
 286 continuous flow mode. Data were expressed as indicated above.

### 287 *Calculations*

289 The proportion of newly assimilated C in the bulk biomass, SS and starch of the  
 290 different fractions of trees grown in  $^{13}\text{C}$ -depleted conditions ( $F_{\text{Cnew}}$ ) was calculated  
 291 using the following equation (Nogués et al. 2004):

$$292 \quad F_{\text{Cnew}} = \frac{\delta^{13}\text{C}_{\text{Sample}} - \delta^{13}\text{C}_{\text{Ambient}}}{\delta^{13}\text{C}_{\text{Grass}} - \delta^{13}\text{C}_{\text{Ambient}}}$$

293 Where  $\delta^{13}\text{C}_{\text{Sample}}$  was the isotopic composition in the sample;  $\delta^{13}\text{C}_{\text{Ambient}}$  was the natural  
 294 baseline  $\delta^{13}\text{C}$  value for the bulk biomass, SS or starch of a given fraction of each species  
 295 calculated from fractions collected from ambient trees in November 2008 (in the case of  
 296 the bulk biomass) or, in the case of SS and starch, by applying an enrichment of 1.71  
 297 and 2.02, respectively, owing to values reported in the literature (Badeck et al. 2005);  
 298 and  $\delta^{13}\text{C}_{\text{Grass}}$  was the average of the  $\delta^{13}\text{C}$  values obtained for *Lolium perenne* plants that  
 299 were grown from seed within the polytunnel and hence represented C arising from

1  
2  
3 300 current assimilation. We assumed that the discrimination against  $^{13}\text{C}$  during  
4  
5 301 photosynthesis would be the same in both the grasses and the trees, because the grasses  
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7 302 were grown under the same conditions as the trees and had no water stress.  
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#### 11 304 *Statistical analyses*

12 305 We used univariate general linear models (glm) to analyse for differences in biomass  
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14 306 and NSC-allocation between species, fractions and treatments. Short-term effects of  
15  
16 307 simulated browsing on biomass and NSC-allocation of trees harvested in April 2008  
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18 308 (before the  $^{13}\text{C}$ -labelling experiment) and the effects of simulated browsing on tree  
19  
20 309 growth, architecture, leaf morphology, biomass, NSC and % new C-allocation (in bulk  
21  
22 310 biomass, SS and starch) and NSC concentrations in the year after clipping (trees  
23  
24 311 harvested in August 2008) were evaluated by glms with species, fractions and  
25  
26 312 treatments as fixed effects. Treatment effects were further tested within fractions and  
27  
28 313 species using one-way ANOVAs. The initial length (i.e. distance from the base to the  
29  
30 314 tip of the tree) of trees at the beginning of the experiment was included as a covariate in  
31  
32 315 all analyses. Starch and NSC concentrations were angularly transformed to meet  
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34 316 normality and homogeneity in variance assumptions. Analyses were done with SPSS  
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36 317 Statistics 17.0.  
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## 47 319 **Results**

### 48 320 *Short-term effects of clipping on biomass and NSC allocation*

49 321 At the beginning of the labelling experiment (i.e. seven months after the application of  
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51 322 treatments), trees subjected to two successive clipping events were significantly smaller  
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53 323 than control trees and showed a significant reduction in the biomass of the youngest  
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55 324 shoot cohort, i.e. that formed in 2007 and directly affected by clipping, but larger main  
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3 325 stem biomass (particularly in birch, Table 1, Table S1 available as Supplementary Data  
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5 326 at Tree Physiology Online). Trees were still dormant and leafless at this time, and no  
6  
7 327 other significant differences were observed in the allocation to different tree fractions in  
8  
9 328 either of the two species. Differences in allocation to different plant components were  
10  
11 329 quantified between birch and oak trees: birch allocated significantly more biomass to  
12  
13 330 above-ground fractions like young and main stems, while oak had significantly more  
14  
15 331 biomass in coarse roots (Table 1, Table S1). However, no significant treatment x  
16  
17 332 species interaction was found, indicating that clipping did not lead to a different  
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19 333 response in biomass allocation between species in the short-term.  
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23  
24 334 Clipped trees of both species harvested in April 2008, seven months after the  
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26 335 application of treatments, showed significantly higher starch concentrations in young  
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28 336 stems (Tables S1 and S2, available as Supplementary Data at Tree Physiology Online).  
29  
30 337 However, NSC pools were decreased in the youngest shoot cohort, showing the  
31  
32 338 significant effect of clipping on the biomass reduction of this cohort for both species  
33  
34 339 (Tables S1 and S3, available as Supplementary Data at Tree Physiology Online). Starch  
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36 340 and SS pools in main stems were significantly larger in clipped trees of both species  
37  
38 341 (Tables S1 and S3, available as Supplementary Data at Tree Physiology Online). This  
39  
40 342 was not due to increased NSC concentrations (Tables S1 and S2, available as  
41  
42 343 Supplementary Data at Tree Physiology Online), but to higher allocation of biomass to  
43  
44 344 main stems in clipped trees (Tables 1 and S1 available as Supplementary Data at Tree  
45  
46 345 Physiology Online). Overall, birch trees had higher SS concentrations, while oaks  
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48 346 showed up to three times higher starch concentrations, particularly in coarse roots  
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50 347 (Tables S1 and S2 available as Supplementary Data at Tree Physiology Online).  
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3 349 *Changes in growth, architecture, leaf morphology, biomass and NSC allocation one*  
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5 350 *year after clipping*  
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7 351 Measurements taken at the end of the first year after the application of clipping  
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9 352 treatments (trees harvested in August 2008), indicated a lower compensating ability in  
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11 353 birch than in oak (Table 2). Although the effect of clipping on tree height was not  
12  
13 354 significant in the general model ( $F = 2.5$ ,  $P = 0.134$ ), significant treatment effects arose  
14  
15 355 in birch when both species were analysed separately, with clipped trees being  
16  
17 356 significantly shorter than controls (Table 2). Clipped *B. pubescens* trees also had fewer  
18  
19 357 branches, terminal shoots and short shoots than controls, indicating that significant  
20  
21 358 effects on the architecture of clipped trees remained measureable even one year after  
22  
23 359 clipping. On the contrary, regrowth of *Q. petraea* saplings completely compensated for  
24  
25 360 height and branching differences, with clipped trees showing only a marginally  
26  
27 361 significant smaller number of terminal shoots than control trees after one year (Table 2).  
28  
29 362 As regards the morphology of leaves, we observed no significant differences in  
30  
31 363 individual leaf area and weight or the SLA of clipped and control trees of both species  
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33 364 (Table 2).

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39 365 There were no significant differences in biomass allocation to leaves and coarse  
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41 366 roots of both species in trees harvested one year after clipping, but clipped trees of both  
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43 367 birch and oak allocated significantly more biomass to current-year stems than control  
44  
45 368 trees (Fig. 2). In oak, clipped trees showed more biomass allocation to the main stems  
46  
47 369 and a sharp reduction in biomass allocation to fine roots, which were reduced by 45% in  
48  
49 370 relation to control trees (Fig. 2). Such reduction in fine root biomass led to a significant  
50  
51 371 decrease in the root:shoot ratio of clipped oaks, not observed in birch (Table 2). At the  
52  
53 372 end of the first growing season after clipping, the significant reduction of shoots formed  
54  
55 373 in 2007 observed at the beginning of the C-labelling experiment in both species (Table  
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3 374 1) was only noticeable in birch, and differences between treatments in oak were no  
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5 375 longer significant (Fig. 2). There were no significant differences in total plant biomass  
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7 376 between treatments, either in the general model ( $F = 3.1$ ,  $P = 0.098$ ) or when species  
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9 377 were analysed separately ( $F = 2.3$ ,  $P = 0.169$  and  $F = 1.3$ ,  $P = 0.280$  in birch and oak,  
10  
11 378 respectively).

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14 379 Differences between control and clipped trees in NSC pools of different plant  
15  
16 380 fractions mimicked results for biomass allocation. Higher SS (and also starch in the case  
17  
18 381 of oak) pools were found in current-year stems of clipped trees of both species (Fig. 3).  
19  
20 382 Contrastingly, NSC pools were lower in one-year old stems of clipped birch trees (Fig.  
21  
22 383 3). Clipped oak trees showed also a trend for higher net allocation of SS to main stems,  
23  
24 384 while both starch and SS pools were significantly reduced in their fine roots (Fig. 3).

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26 385 The differences observed in NSC pool allocation were not driven by changes in  
27  
28 386 NSC concentrations, which remained similar for both SS and starch across treatments  
29  
30 387 and fractions, except for SS concentrations in the leaves of clipped birch trees, which  
31  
32 388 were lower than those of control trees (Table 3). In any case, the lower SS  
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34 389 concentrations of the leaves of clipped birch trees did not result in significantly different  
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36 390 SS pools between control and clipped birch trees (Fig. 3, Table 3).

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44 392 *Changes in the net allocation of newly fixed C to different plant fractions and the soil*  
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46 393 *during the recovery of clipped birch and oak trees*

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49 394 The two species showed very different responses in their  $\delta^{13}\text{C}$  isotopic composition  
50  
51 395 after labelling and clipping (Table S4 available as Supplementary Data at Tree  
52  
53 396 Physiology Online). In general, birch allocated significantly more new C to biomass  
54  
55 397 than oak, pointing to a lower reliance on C-stores and a higher C-fixing ability of birch  
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57 398 than oak (Fig. 4, Table 4). Across species and treatments, the fractions receiving  
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3 399 proportionally more newly fixed C were rapidly growing ones, including, in descending  
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5 400 order, leaves, current-year stems, young (one and two-year-old) stems and fine roots  
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7 401 (Fig. 4). Despite being winter deciduous, the leaves of both species were mainly built on  
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9 402 newly fixed C, which accounted for 80% of the bulk leaf biomass in birch and over 65%  
10  
11 403 in oak (Fig. 4).

12  
13  
14 404 Clipped trees allocated significantly more newly-fixed C to the different  
15  
16 405 fractions than controls (Table 4, Fig. 4), but results were very different depending on the  
17  
18 406 species (Fig. 4, Table S5 available as Supplementary Data at Tree Physiology Online).  
19  
20 407 This explains why all interaction terms in the general model were significant (Table 4).  
21  
22 408 In general, clipped trees tended to allocate more new C into fast growing fractions like  
23  
24 409 young stems and fine roots than control trees (Fig. 4, Table S5 available as  
25  
26 410 Supplementary Data at Tree Physiology Online), but when both species were analysed  
27  
28 411 separately, effects were only significant in oak (Fig. 4). In the year after clipping,  
29  
30 412 clipped *Q. petraea* trees allocated significantly more new C than controls to all fractions  
31  
32 413 but the main stems (Fig. 4). Contrastingly, birch trees showed no differences in the  
33  
34 414 allocation of new C to different fractions between control and clipped trees, except for  
35  
36 415 the main stems, where control trees received a larger proportion of new C (Fig. 4).

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38  
39 416 The rhizosphere soil collected underneath both study species showed more  $^{13}\text{C}$ -  
40  
41 417 depleted values as compared to the bulk soil ( $F = 118.6$ ,  $P < 0.001$ ; Fig. 5), indicating  
42  
43 418 that plant roots significantly altered the  $\delta^{13}\text{C}$  signature of the soil in direct contact with  
44  
45 419 them.  $\delta^{13}\text{C}$  values of the rhizosphere soil collected underneath birch trees were more  
46  
47 420 depleted than those of oak, potentially indicating a larger amount of labelled-C  
48  
49 421 transferred to the rhizosphere in this species ( $F = 6.5$ ,  $P = 0.016$ ; Fig. 5). However, no  
50  
51 422 significant clipping effects were observed in the  $\delta^{13}\text{C}$  signature of bulk and rhizosphere  
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3 423 soil in both species (Fig. 5). This indicates the loss of newly-fixed C from roots was  
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5 424 similar in clipped and control treatments of both species.  
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10 426 *Differences in the allocation of new C to NSC between clipped and control trees*

11  
12 427 Birch allocated more new C to SS than oak in all fractions and also significantly more  
13  
14 428 new C to starch, particularly in fast turnover fractions like leaves, young stems and fine-  
15  
16 429 roots, while both species showed similar new C-allocation to starch in coarse roots and  
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18 430 main stems (Table 4, Fig. 6, Table S6 available as Supplementary Data at Tree  
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20 431 Physiology Online). In general, clipping had no significant effect on the allocation of  
21  
22 432 newly fixed C to storage (both SS and starch) during the next season after clipping  
23  
24 433 (Table 4). However, in the case of the allocation to starch the response varied depending  
25  
26 434 on the species and the fraction and, consequently, the interaction terms between species  
27  
28 435 and treatment and the full interaction term were significant (Table 4). Simplified models  
29  
30 436 run separately per species and fraction indicated that, in birch, clipped trees allocated  
31  
32 437 significantly less C to starch in leaves and main stems (the latter marginally significant  
33  
34 438 only) and marginally higher C to SS in current-year stems than control ones (Fig. 6).  
35  
36 439 Contrastingly, clipped oaks showed a trend for higher allocation of newly fixed C to  
37  
38 440 starch in current-year and main stems (Fig. 6), but results were only marginally  
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40 441 significant.  
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49 443 **Discussion**

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51 444 In accordance with our first hypothesis, our results indicate that clipped birch and oak  
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53 445 trees shifted their C-allocation patterns above-ground as a means to recover from  
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55 446 defoliation. However, contrary to our expectations, such increased allocation to current-  
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57 447 year stems and leaves did not entail reductions in the allocation to the rhizosphere. As  
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3 448 initially expected, *B. pubescens* and *Q. petraea* showed differences in their vulnerability  
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5 449 and recovery strategies to clipping, the ring-porous species being less vulnerable than  
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7 450 the diffuse porous. These contrasting patterns could be explained by differences in their  
8  
9 451 C cycling after clipping. Defoliated oaks showed a more efficient recovery of their  
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11 452 canopy, which was supported by a larger allocation of new C into biomass, particularly  
12  
13 453 aboveground. However, this was associated with large decreases in the fine root  
14  
15 454 biomass of clipped oak trees. Although in both species clipped trees recovered NSC  
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17 455 pools faster than growth, the allocation of  $^{13}\text{C}$ -labelled photo-assimilates into starch and  
18  
19 456 SS was not increased as compared to controls. This indicates that, contrary to our  
20  
21 457 second hypothesis, no preferential allocation into NSC occurred during the first year  
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23 458 after clipping.  
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30 460 *Trees recovered from defoliation by increasing C allocation aboveground but*  
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32 461 *maintaining allocation to the rhizosphere*  
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35 462 Clipping led to a rapid reduction in tree growth (Palacio et al. 2011) and also in biomass  
36  
37 463 and NSC pools in current-year stems (the fraction directly affected by clipping  
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39 464 treatments). Over the course of the first year after clipping, trees managed to recover  
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41 465 initial differences in main stem diameter, total plant biomass and, in the case of oaks,  
42  
43 466 also tree height (Table 2). This was mainly a result of shifting allocation above-ground,  
44  
45 467 with increased allocation to current-year stems (almost double to that of control trees in  
46  
47 468 oak) and producing similar leaf biomass to controls (Fig. 2). In oak, these results were  
48  
49 469 consistent for both biomass allocation as a whole and when the proportion of new C  
50  
51 470 allocated to bulk biomass was considered (Fig. 4). The ability of trees to recover from  
52  
53 471 defoliation by increased above-ground allocation is a well-known phenomenon (Eyles  
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55 472 et al. 2009; Quentin et al. 2011). Such increased allocation may be achieved by a  
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3 473 combination of shifts in architecture, leaf morphology and C-allocation patterns (Eyles  
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5 474 et al. 2009).

6  
7 475 The removal of apical buds by browsing, clipping or defoliating insects  
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10 476 frequently leads to a decrease in apical dominance (due to changes in auxin fluxes,  
11  
12 477 Teichmann and Muhr 2015) with subsequent increases in lateral branch growth  
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14 478 (Haukioja et al. 1990; Wilson 1993). Clipped trees in our study showed a lower number  
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16 479 of terminal shoots, but increased branching was not detected in either of the two study  
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18 480 species. Clipped oaks showed similar lateral branch numbers to control trees, while  
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20 481 clipped birch trees had less lateral branches than controls. However, birch trees showed  
21  
22 482 a dramatic decrease in the number of short shoots produced after clipping (Table 2,  
23  
24 483 Palacio et al. 2011), which may be a direct consequence of the decreased apical  
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26 484 dominance after clipping (Haukioja et al. 1990). In addition, increases in the proportion  
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28 485 of leaves per bud (Millard et al. 2001) or in the foliage to wood ratio (Mizumachi et al.  
29  
30 486 2004) have been reported as mechanisms to increase above-ground allocation in clipped  
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32 487 trees. This was not the case in the trees included in this experiment, which showed  
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34 488 similar leaf biomass to control trees but increased current-year stem biomass. It seems,  
35  
36 489 therefore, that clipped trees in our experiment maximized the recovery of the canopy,  
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38 490 increasing investment into new stems while keeping a similar allocation to foliage. In  
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40 491 clipped birch trees, the decrease in short shoot number (likely in favour of long shoot  
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42 492 development), may be a mechanism to recover canopy spread and renewal of bud  
43  
44 493 numbers since, in this species, renewal buds are mostly borne in the long shoots  
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46 494 (MacDonald et al. 1984).

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48  
49 495 Several previous studies have detected shifts in leaf morphology after defoliation  
50  
51 496 to compensate losses in the C-assimilating capacity of the canopy, frequently leading to  
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53 497 increased individual leaf area and weight and increased SLA (Fuenzalida et al 2019;  
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3 498 Millard et al. 2001; Piper and Fajardo 2015; Quentin et al. 2011; Trumble et al. 1993).  
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5 499 We did not detect any significant differences in leaf morphology between clipped and  
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7 500 control trees of either study species. Discrepancies with previous studies may be related  
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9 501 to differences in the type of disturbance applied and in the duration of experiments. For  
10  
11 502 example, Fuenzalida et al. (2019), Piper and Fajardo (2015) and Trumble et al. (1993)  
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13 503 evaluated responses after defoliating insects or treatments simulating defoliation by  
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15 504 arthropods, which may elicit a very different response by trees than clipping (Haukioja  
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17 505 et al. 1990). Further, Millard et al. (2001) and Quentin et al. (2011) applied clipping in  
18  
19 506 spring and measured the effects on leaf morphology at the end of the same growing  
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21 507 season, while in our study effects on leaf morphology were recorded at the end of the  
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23 508 next growing season after clipping, i.e. a much longer duration. Similarly, Eyles et al.  
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25 509 (2009) carried out a shorter duration experiment and did not detect any significant  
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27 510 effects on individual leaf area of *Eucalyptus globulus* five months after 40% defoliation.  
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33 Our results show that the increased above-ground allocation of clipped trees was  
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35 512 largely supported by currently-fixed (new) C, particularly in oak (Fig. 4). Increases in  
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37 513 photosynthetic rate have been repeatedly reported in defoliated trees (e.g. Pinkard et al.  
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39 514 1998; Vanderklein and Reich 1999). Although we did not measure photosynthetic rates  
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41 515 in our study, clipped oak trees showed higher new-C-allocation to bulk biomass,  
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43 516 compatible with increased C-fixing ability and with decreased respiratory losses (see  
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45 517 below). Both in control and clipped trees of both species, new C was preferentially  
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47 518 allocated to actively growing fractions, like leaves, young stems and fine roots.  
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51 519 Although clipping induced increased C-allocation above-ground, our results  
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53 520 showed no significant effects of clipping on net deposition to soil in either of the two  
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55 521 species analyzed. Frost and Hunter (2008) obtained similar results in red oak (Frost and  
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57 522 Hunter 2008) and suggested that rhizodeposition might be a tightly controlled process  
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3 523 buffered against damage-induced shifts in C-allocation. Both species had a significant  
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5 524 effect on the  $\delta^{13}\text{C}$  isotopic composition of the soil, indicated by the depletion in  $^{13}\text{C}$   
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7 525 detected in rhizosphere vs. bulk soils. Such an effect was larger in birch than oak, likely  
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9 526 in relation to the larger C-fixing ability of the former. Consequently, trees were able to  
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11 527 impose changes in the C dynamics of soils, but such effects were not modified by  
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14 528 clipping.

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19 530 *B. pubescens trees were more severely affected than oaks by clipping.*

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21 531 In agreement with the predictions by Foster (2017), the ring-porous species, *Q. petraea*,  
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23 532 was more efficient in recovering the biomass lost by clipping than the diffuse-porous, *B.*  
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25 533 *pubescens*. Clipped birch trees showed lower height and altered architecture (reduced  
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27 534 number of branches, terminal and lateral shoots) as compared to controls. Also, while  
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29 535 biomass losses in stems formed in 2007 (the cohort directly affected by clipping  
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31 536 treatments) were no longer significant at the end of the 2008 growing season in oaks,  
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33 537 they were still noticeable in birch. Foster (2017) hypothesized that ring-porous species  
34  
35 538 would be more resistant to spring defoliation than diffuse-porous ones owing to their  
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37 539 earlier wood phenology and increased C-stores (Barbaroux and Bréda 2002; Barbaroux  
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39 540 et al. 2003; Dougherty et al. 1979). We did not measure wood phenology in this study,  
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41 541 but it seems likely that this might have had an effect on the differential responses of  
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43 542 both species. In our experiment, clipping consisted of shoot removal in early July and  
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45 543 early September 2007, after the first and second flushes of shoot growth were finished.  
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47 544 If the differences in wood growth phenology between ring-porous and diffuse-porous  
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49 545 adult trees can be applied to saplings, oaks would be expected to have started wood  
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51 546 growth at least two weeks prior to the first defoliation event, while birch trees would be  
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53 547 just starting (Foster 2017). Foster (2017) further hypothesized that such differences in  
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3 548 damage due to wood growth phenology would entail a differential decrease in NSC  
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5 549 stores, which would be more severely decreased in diffuse-porous trees and further  
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7 550 exacerbated by their lower C-storage capacity. Our results do not confirm this  
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9 551 prediction, since both species were equally able to recover NSC pools to the same level  
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11 552 as controls on the same year of clipping (except for the shoot cohort directly affected by  
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13 553 treatments). Similar fast recovery of NSC stores in defoliated trees has been previously  
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15 554 reported (Palacio et al. 2008; Piper et al. 2015; Puri et al. 2015).

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17 555         Instead, our results show that the differential recovery ability of birch and oak  
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19 556 trees after clipping could be, at least partly, explained by the different effects of clipping  
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21 557 on their C-cycling, including differences in C-allocation. In accordance with previous  
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23 558 studies, oaks showed a larger reliance on storage than birch trees to support new growth  
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25 559 (Barbaroux et al. 2003; but see Palacio et al. 2011). This increased ability to re-mobilise  
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27 560 C-stores could have been crucial to support the re-growth of clipped oaks, at least  
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29 561 initially. Several previous studies have reported a positive relationship between NSC  
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31 562 storage and the re-growth ability of defoliated trees (Fierravanti et al. 2019; Kays and  
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33 563 Canham 1991; Luostarinen and Kauppi 2005). However, clipped oak trees invested  
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35 564 significantly less “old” C (and proportionally more “new” C) in their new growth than  
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37 565 control trees (Fig. 4). Consequently, while clipped oaks recovered to a larger extent than  
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39 566 clipped birch trees in our experiment, this was not linked to increased total NSC  
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41 567 remobilisation as measured at the end of the growing season.

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43 568         The increased allocation of new C in clipped oaks to support re-growth as  
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45 569 compared to controls illustrates a shift in C cycling after damage resulting in  
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47 570 significantly more new C being allocated to most fractions in clipped vs. control oaks,  
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49 571 an effect not observed in birch. Such a change can be the result of increased C-fixation,  
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51 572 but also of decreased losses by respiration or rhizosphere allocation. Differences in C  
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3 573 allocation to the rhizosphere were not significant among treatments (see above).  
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5 574 However, we cannot rule out the possibility that the increased allocation of new C  
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7 575 assimilates in oak was (at least partly) due to reduced respiratory losses, particularly in  
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9 576 relation to the drastic reduction in fine root biomass detected in clipped trees of this  
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11 577 species. Accordingly, the increased investment into above-ground components in oak  
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13 578 was associated with a reduced production of fine roots of 45% during the year after  
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15 579 clipping. These changes were not observed in birch, which maintained similar biomass  
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17 580 allocation below-ground between control and clipped trees. Increased fine root mortality  
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19 581 is a frequently reported process in defoliated trees (Frost and Hunter 2008; Tuomi et al.  
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21 582 1990; Vanderklein and Reich 1999; but see Endrulat et al. 2016; Kosola et al. 2001).  
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23 583 Tuomi et al. (1990) suggested that the reductions in fine root biomass after defoliation  
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25 584 could vary largely depending on the relative root biomass, the degree of reserve  
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27 585 depletion and the compensatory C gain of trees. All these three factors likely differed in  
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29 586 the two study species, which could explain their contrasting response. Oaks have  
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31 587 relatively high root:shoot ratios (Shaw 1974) as was the case also in this experiment  
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33 588 (Table 1). This means an increased non-productive biomass to support during re-growth  
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35 589 (Tuomi et al. 1990). The higher storage pool may not have been sufficient to recover  
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37 590 above-ground losses and maintain a large root biomass in oak (Tuomi et al. 1990).  
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49 592 *Clipping did not result in preferential C-allocation to storage in the long-term*

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51 593 Our study did not detect temporal differences in NSC concentrations in the different  
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53 594 organs of clipped and control trees throughout the first year of recovery, but NSC  
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55 595 concentrations of clipped trees of both species reached similar levels to those of control  
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57 596 trees by the end of the first growing season. This indicates that decreases in NSC stores  
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59 597 due to canopy re-growth, if any, were short-lived and fully compensated within less  
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3 598 than one year. Similar results have been previously reported in the literature (e.g.  
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5 599 Palacio et al. 2012; Puri et al. 2015; Wiley et al. 2013). In our experiment, the  
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7 600 replenishment of stores was likely supported by an increased C-fixing ability in clipped  
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9 601 trees, particularly in oak, as denoted by their higher new-C-allocation to bulk biomass.

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12 602 Despite NSC concentrations of clipped trees were rapidly restored to even higher  
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14 603 levels than control trees (Tables S2, S3), we did not detect a significant increase in new  
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16 604 C-allocation to storage in clipped trees in the first year after clipping. The only  
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18 605 significant effect of clipping on new C-allocation was a reduction in allocation to SS in  
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20 606 leaves of clipped birches. Wiley et al. (2017b; 2013) suggested that the growth of  
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22 607 defoliated trees would be largely limited by C-availability, first by the decrease in leaf  
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24 608 area directly related to defoliation, and then by a prioritized allocation to storage over  
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26 609 growth to secure tree survival under future potential defoliation events. They argued  
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28 610 that the fact that NSC stores were replenished to control levels did not necessarily mean  
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30 611 tree growth was not limited by C-availability, since prioritized allocation to storage over  
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32 612 growth could still proceed (Wiley and Helliker 2012). Two recent experiments have  
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34 613 experimentally demonstrated that NSC concentrations can be maintained to control  
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36 614 levels over periods of C-limitation by preferential allocation of C into storage, calling  
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38 615 for a cautious use of NSC concentrations to predict the C-status of trees (Weber et al.  
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40 616 2019; 2018). In both experiments, trees subjected to low or no illumination were  
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42 617 progressively C-deprived, reaching minimum SS and starch thresholds below which tree  
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44 618 survival was impaired. In both cases, re-illumination resulted in a period of reduced  
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46 619 growth and refilling of NSC stores up to a certain threshold. These results indicate that  
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48 620 prioritized allocation to storage over growth does occur in C-starved trees (with very  
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50 621 low NSC levels), and that such prioritization is arrested once a certain level of recovery  
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58 622 of NSC is achieved.  
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3 623 In contrast to the experiments in Weber et al. (2019; 2018), our trees were not C-  
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5 624 starved. We did not find the very significant depletion of NSC reported by these  
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7 625 previous studies (Weber et al. 2019; 2018). Consequently, our trees probably did not  
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9 626 prioritize allocation to NSC over growth. Starch and SS concentrations of defoliated  
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11 627 trees are normally not depleted below the C-starvation thresholds detected by Weber et  
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13 628 al. (2019; 2018), even after severe treatments (e.g. Kays and Canham 1991; Palacio et  
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15 629 al. 2012; Vanderklein and Reich 1999, but see Kosola et al. 2001; Puri et al. 2015). This  
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17 630 seems to indicate C-limitation after defoliation in trees is normally short-lived and of  
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19 631 low magnitude.  
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## 25 633 **Conclusions**

26  
27 634 Our results show that clipping triggers a shift in biomass allocation aboveground  
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29 635 favouring the recovery of the canopy both in oak and birch trees. However, such a shift  
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31 636 does not entail a decrease in C-allocation to the rhizosphere, which seems to be a tightly  
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33 637 regulated process. Future research on the mechanisms behind such tight regulation  
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35 638 would greatly contribute to our understanding on the effects of defoliation on tree C-  
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37 639 cycling and its impact on belowground processes. The observed differences in the  
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39 640 recovery strategies of the two study species could have potential implications for their  
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41 641 vulnerability under different browsing frequencies. The ring porous species, *Q. petraea*,  
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43 642 showed a faster recovery of its canopy after clipping than the diffuse porous, *B.*  
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45 643 *pubescens*. However, this came at the cost of a marked decrease in the fine root biomass  
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47 644 of oak, which raises questions on the potential consequences for the nutrition of the tree  
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49 645 and its vulnerability to sustained browsing over longer time periods. Finally, despite the  
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51 646 different effect of clipping on the C-allocation of study species, none of them increased  
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3 647 new C allocation to storage one year after damage. This indicates that clipping does not  
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5 648 entail a sustained preventative allocation of C into storage in the long term.  
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10 **Data and Materials Availability**

11  
12 651 Authors agree to make experimental data and materials available to third party academic  
13  
14 652 researchers upon reasonable request.  
15  
16

17 653

18  
19 **Supplementary Data**

20  
21 655 The following supporting documents are provided as additional content:

22  
23 656 - Table S1. Results of glms on the effect of clipping on the biomass (BM), and soluble  
24  
25 657 sugars (SS) and starch pools and concentrations of the different fractions of *B.*

26  
27  
28 658 *pubescens* and *Q. petraea* trees harvested at the beginning of the  $\delta^{13}\text{C}$ -labelling  
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30 659 experiment in April 2008.

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32  
33 660 - Table S2. Soluble sugars (SS) and starch concentrations ( $\text{mg g}^{-1}$ ) in the different plant  
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35 661 fractions of *B. pubescens* and *Q. petraea* trees harvested at the beginning of the  $\delta^{13}\text{C}$ -  
36  
37 662 labelling experiment in April 2008.

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39  
40 663 - Table S3. Soluble sugars (SS) and starch pools (as % of the total SS or starch pool in  
41  
42 664 the plant) in the different plant fractions in *B. pubescens* and *Q. petraea* trees harvested  
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44 665 at the beginning of the  $\delta^{13}\text{C}$ -labelling experiment in April 2008.

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46  
47 666 - Table S4. Isotopic composition ( $\delta^{13}\text{C}$ ; ‰) of the different fractions of *B. pubescens*  
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49 667 and *Q. petraea* saplings harvested in August 2008.

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51 668 - Table S5. Results of glms on the effect of “Species” and “Treatment” on the allocation  
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53 669 of new C to bulk biomass of the different fractions of *B. pubescens* and *Q. petraea* trees  
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55 670 harvested in August 2008.  
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3 671 - Table S6. Results of glms on the effect of “Species” and “Treatment” on the allocation  
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5 672 of new C to soluble sugars (SS) and starch in the different fractions of *B. pubescens* and  
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7 673 *Q. petraea* trees harvested in August 2008.  
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#### 11 675 **Conflict of interest**

12  
13  
14 676 None declared.  
15  
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17 677

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30 683

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36  
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39 687 anonymous Reviewers for useful comments on an earlier version of the manuscript.  
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41

42 688

#### 43 689 **Authors' Contributions**

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46 690 SP, PM, AH and EP designed the study; SP, EP and PM implemented continuous  $\delta^{13}\text{C}$ -  
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48 691 labelling. SP, EP, PM, MM, GL, SN and AAR run carbohydrate and stable isotope  
49  
50 692 analyses. SP, PM and SN analysed data. All authors interpreted results. SP wrote the  
51  
52 693 manuscript receiving revisions from all authors.  
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3 **912 Figure legends**  
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5 **913 Fig. 1.** Experimental design with the indication of the main treatments (clipping – red  
6 arrows-,  $^{13}\text{C}$ -labelling), growth measurements (blue arrows) and harvests (black  
7 arrows) applied to *B. pubescens* and *Q. petraea* saplings.  
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10 **915**  
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12 **916 Fig. 2.** Biomass allocation to different tree fractions in clipped and control trees of *B.*  
13 *pubescens* and *Q. petraea* harvested in August 2008 (ca. one year after clipping).  
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15 Average values are shown for each fraction. L = Leaves, 0-St = current year stems, 1-St  
16 = one-year-old stems, 2-St = two-year-old stems, MS = main stems, CR = coarse roots,  
17 FR = fine roots. Significant differences between treatments within species are indicated  
18 by asterisks: \* =  $P < 0.10$ , \*\* =  $P < 0.05$ .  
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21 **922 Fig. 3.** Differences between clipped (grey bars) and control (black bars) *B. pubescens*  
22 and *Q. petraea* saplings in the allocation of SS and starch pools to different plant  
23 fractions one year after clipping (trees harvested in August 2008). Asterisks denote  
24 significant differences between treatments within a given species at  $\alpha = 0.10$  (\*) and  $\alpha =$   
25 0.05 (\*\*).  
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28 **927 Fig. 4.** Differences between clipped (grey bars) and control (black bars) *B. pubescens*  
29 and *Q. petraea* saplings in the allocation of newly fixed C to the different plant organs  
30 one year after clipping (trees harvested in August 2008). Asterisks denote significant  
31 differences between treatments within a given species at  $\alpha = 0.05$ .  
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34 **931 Fig 5.** Isotopic composition ( $\delta^{13}\text{C}$ ; ‰) of the rhizosphere and bulk soil collected  
35 underneath control (white dots) and clipped (black dots) *B. pubescens* and *Q. petraea*  
36 trees harvested in August 2008 (ca. one year after clipping). No significant differences  
37 between treatments were detected at  $\alpha = 0.05$ .  
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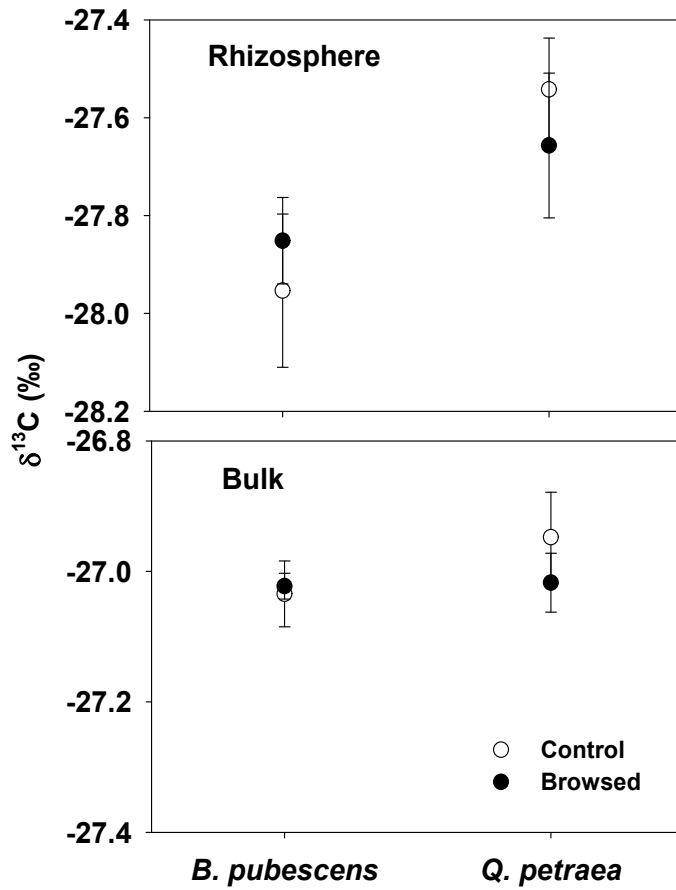
40 **935 Fig 6.** Differences between control (black bars) and clipped (grey bars) *B. pubescens*  
41 and *Q. petraea* saplings in the allocation of newly fixed C to SS and starch to different  
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3 937 plant fractions on the year after clipping (trees harvested in August 2008). Asterisks  
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5 938 denote significant differences between treatments within a given species at  $\alpha = 0.10$  (\*),  
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8 939 and  $\alpha = 0.05$  (\*\*).  
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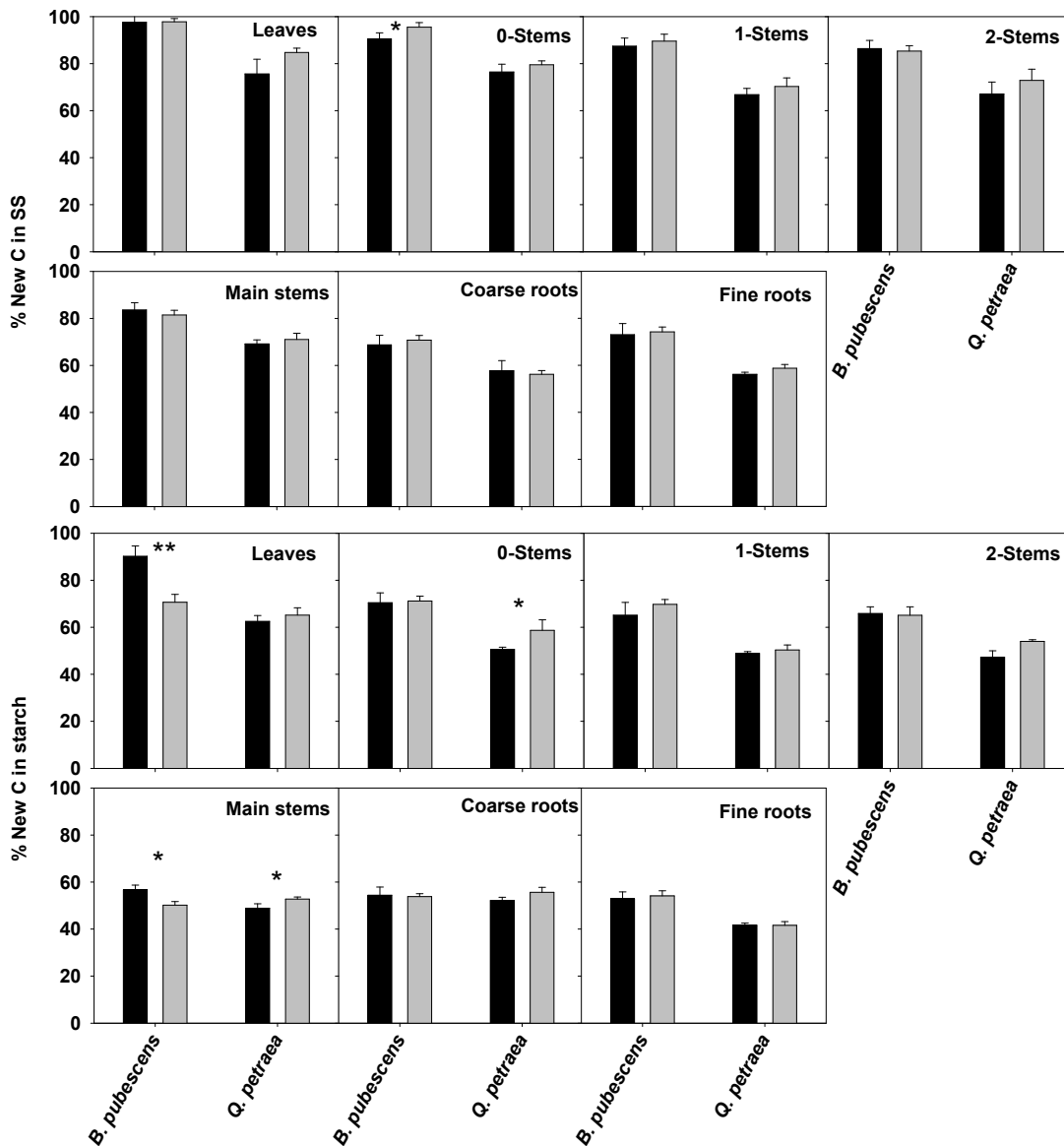
For Peer Review



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**Table 1.** Biomass allocation to the different plant fractions in *B. pubescens* and *Q. petraea* trees harvested at the beginning of the  $\delta^{13}\text{C}$ -labelling experiment in April 2008 (i.e. at the beginning of the first growing season after clipping). Data are means and SE (in parentheses). Significant differences between treatments within species are indicated by asterisks: \* =  $P < 0.10$ , \*\* =  $P < 0.05$ ,  $N = 5$ .

BM allocation (% total plant biomass)	<i>B. pubescens</i>		<i>Q. petraea</i>	
	Clipped	Control	Clipped	Control
One-year-old stems	2.4 (0.3)	9.8 (1.6)**	1.3 (0.3)	4.8 (0.7)**
Two-year-old stems	12.5 (2.8)	14.3 (1.8)	11.1 (2.0)	11.8 (1.5)
Main stems	30.3 (1.8)	23.8 (2.0)*	21.7 (1.5)	18.6 (1.3)
Coarse roots	25.0 (1.9)	24.8 (2.0)	39.6 (3.0)	36.8 (1.6)
Fine roots	16.0 (1.3)	16.6 (3.7)	10.0 (1.2)	13.8 (0.9)



**Table 2.** Results for architectural: tree height (cm), canopy area (m<sup>2</sup>), basal stem diameter (mm), number of branches, number of terminal shoots, number of lateral shoots, number of long shoots, number of short shoots; and morphological: individual leaf area (LA; cm<sup>2</sup>), individual leaf weight (g), and specific leaf area (SLA, cm<sup>2</sup> g<sup>-1</sup>) variables measured in clipped and control saplings of *B. pubescens* and *Q. petraea* before harvest in August 2008 (ca. one year after clipping). Significant differences between treatments within species are highlighted in bold. Asterisks indicate the degree of significance: \* =  $P < 0.10$ ; \*\* =  $P < 0.05$ ; \*\*\* =  $P < 0.001$ ,  $N = 5$ .

Measurements	<i>B. pubescens</i>		<i>Q. petraea</i>	
	Clipped	Control	Clipped	Control
Tree height (cm)	<b>130.9 (8.7)</b>	<b>161.8 (11.3)</b> **	124.8 (6.9)	128.6 (17.4)
Canopy area (m <sup>2</sup> )	2.9 (0.4)	2.5 (0.2)	2.3 (0.4)	2.6 (0.3)
Stem diam (mm)	15.5 (0.3)	15.3 (0.5)	14.3 (0.5)	14.9 (0.5)
No. branches	<b>12.4 (0.7)</b>	<b>25.8 (1.7)</b> ***	7.4 (1.4)	13.2 (2.9)
No. terminal shoots	<b>15.4 (1.6)</b>	<b>38.2 (7.5)</b> **	<b>6.0 (0.8)</b>	<b>12.2 (2.8)</b> *
No. lateral shoots	47.4 (6.9)	61.6 (17.4)	15.4 (1.9)	20.8 (5.0)
No. long shoots	62.8 (7.9)	99.8 (23.9)	21.4 (1.4)	33.0 (6.8)
No. short shoots	<b>20.6 (3.1)</b>	<b>237.0 (59.0)</b> **	0.2 (0.2)	1.4 (0.9)
Root:Shoot ratio	0.52 (0.05)	0.44 (0.07)	<b>0.57 (0.06)</b>	<b>0.81 (0.04)</b> **
Ind. LA (cm <sup>2</sup> )	10.8 (0.8)	9.2 (0.4)	41.2 (5.4)	40.6 (4.9)
Ind. leaf weight (g)	0.04 (0.00)	0.03 (0.00)	0.18 (0.03)	0.19 (0.02)
SLA (cm <sup>2</sup> g <sup>-1</sup> )	250.1 (11.0)	273.9 (20.8)	231.2 (10.0)	219.9 (10.5)

**Table 3.** Soluble sugar (SS) and starch concentrations in the different fractions of clipped (B) and control (C) *B. pubescens* and *Q. petraea* trees harvested in August 2008 (ca. one year after clipping). L = Leaves, 0-St = current year stems, 1-St = one-year-old stems, 2-St = two-year-old stems, MS = main stems, CR = coarse roots, FR = fine roots. Data are means and SE (in parentheses). Significant differences between treatments within a given species at  $\alpha = 0.05$  are indicated by asterisks.

	<i>B. pubescens</i>				<i>Q. petraea</i>			
	SS (mg g <sup>-1</sup> )		Starch (mg g <sup>-1</sup> )		SS (mg g <sup>-1</sup> )		Starch (mg g <sup>-1</sup> )	
	B	C	B	C	B	C	B	C
<b>L</b>	65.2 (3.6)	79.1* (4.0)	69.2 (6.8)	68.0 (3.9)	56.7 (4.8)	50.8 (3.5)	55.0 (9.6)	57.7 (5.1)
<b>0-St</b>	37.9 (1.7)	38.9 (1.4)	40.3 (2.6)	42.9 (3.7)	20.8 (1.1)	21.1 (0.9)	70.2 (8.8)	81.2 (15.9)
<b>1-St</b>	33.4 (3.9)	37.9 (2.5)	36.3 (2.1)	44.9 (6.3)	22.4 (2.2)	22.2 (1.0)	68.2 (10.0)	68.3 (10.4)
<b>2-St</b>	30.9 (2.4)	33.0 (2.0)	43.5 (3.1)	51.6 (9.3)	21.3 (2.2)	19.6 (2.3)	73.6 (13.4)	90.9 (10.9)
<b>MS</b>	23.8 (2.1)	26.3 (1.3)	54.8 (6.4)	43.6 (3.0)	20.0 (2.0)	16.5 (0.7)	107.0 (10.1)	121.3 (14.0)
<b>CR</b>	28.1 (2.5)	30.3 (2.7)	182.2 (14.4)	147.6 (12.7)	27.9 (3.3)	24.3 (1.1)	270.3 (6.4)	266.8 (9.9)
<b>FR</b>	23.4 (1.7)	25.3 (1.6)	85.8 (5.2)	75.0 (8.5)	22.0 (1.5)	19.2 (1.1)	65.7 (5.8)	66.7 (5.6)

**Table 4.** Summary statistics of glm analysis showing the effects of the different factors included in the full model on the allocation of new C to bulk biomass, SS and starch to the different fractions of *B. pubescens* and *Q. petraea* saplings subjected to different clipping treatments and harvested in August 2008 (ca. one year after clipping). F-ratios along with P-values (in parentheses) are shown. Significant differences at  $\alpha = 0.05$  are highlighted in bold.

Model term	Bulk BM	SS	Starch
Initial length	<b>20.4 (&lt; 0.001)</b>	<b>12.3 (0.001)</b>	0.2 (0.641)
Species	<b>170.7 (&lt; 0.001)</b>	<b>212.1 (&lt; 0.001)</b>	<b>105.2 (&lt; 0.001)</b>
Treatment	<b>12.0 (0.001)</b>	1.9 (0.175)	0.1 (0.814)
Fraction	<b>155.6 (&lt; 0.001)</b>	<b>43.2 (&lt; 0.001)</b>	<b>33.8 (&lt; 0.001)</b>
Species * Treatment	<b>17.6 (&lt; 0.001)</b>	1.5 (0.225)	<b>10.0 (0.002)</b>
Species * Fraction	<b>2.8 (0.013)</b>	1.2 (0.314)	<b>6.6 (&lt; 0.001)</b>
Treatment * Fraction	<b>2.5 (0.027)</b>	0.3 (0.957)	<b>2.7 (0.017)</b>
Species * Treat. * Fraction	0.8 (0.562)	0.9 (0.505)	<b>2.5 (0.025)</b>

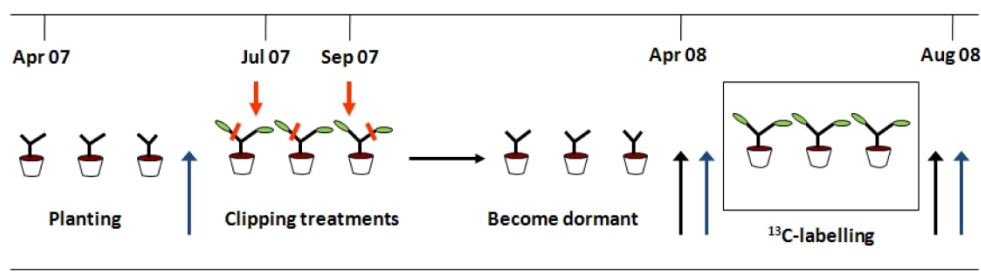


Figure 1

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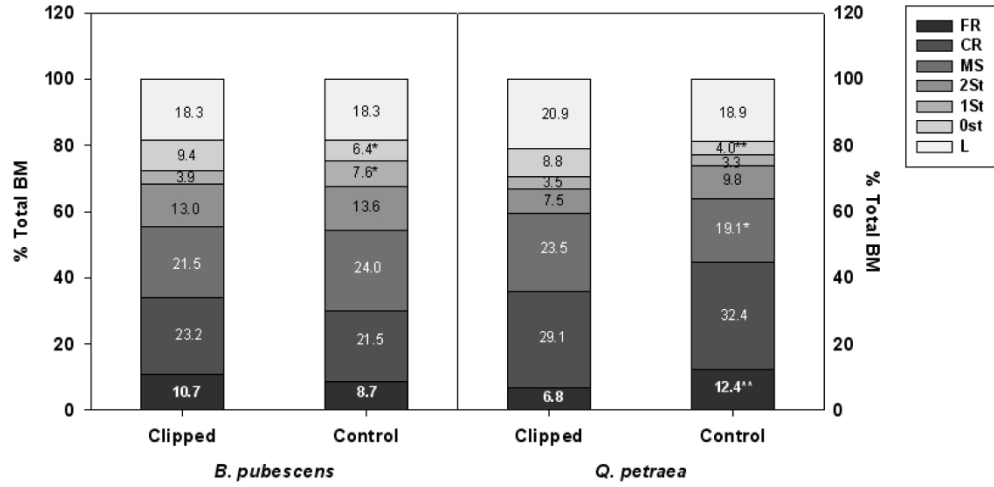


Figure 2

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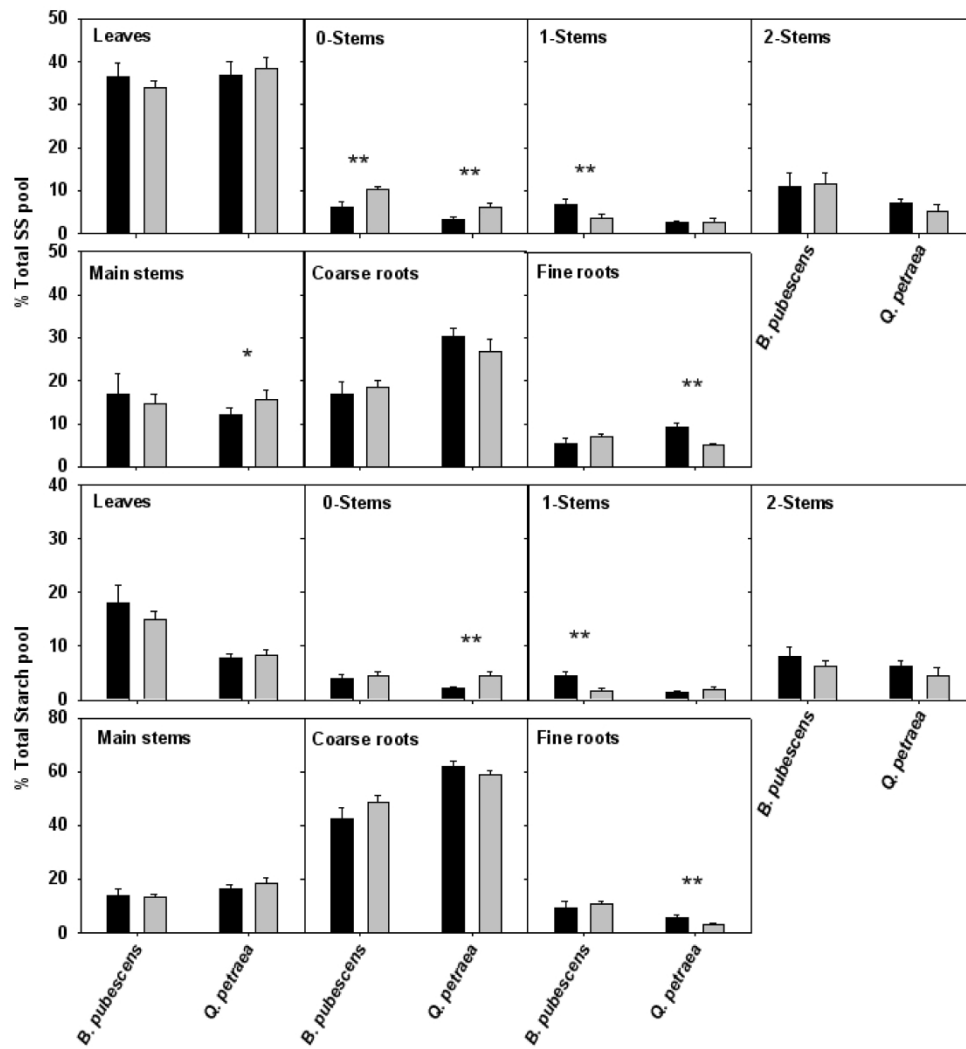


Figure 3

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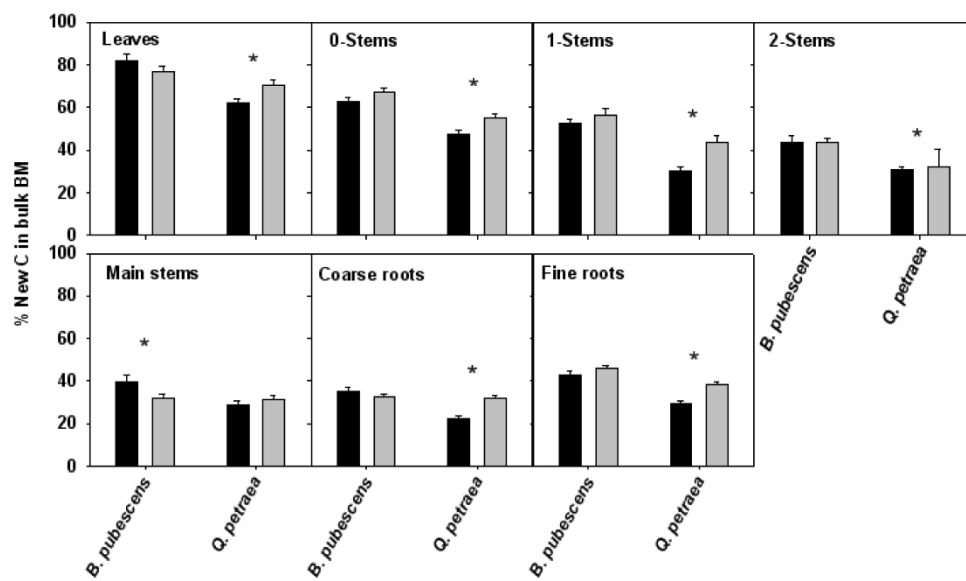


Figure 4

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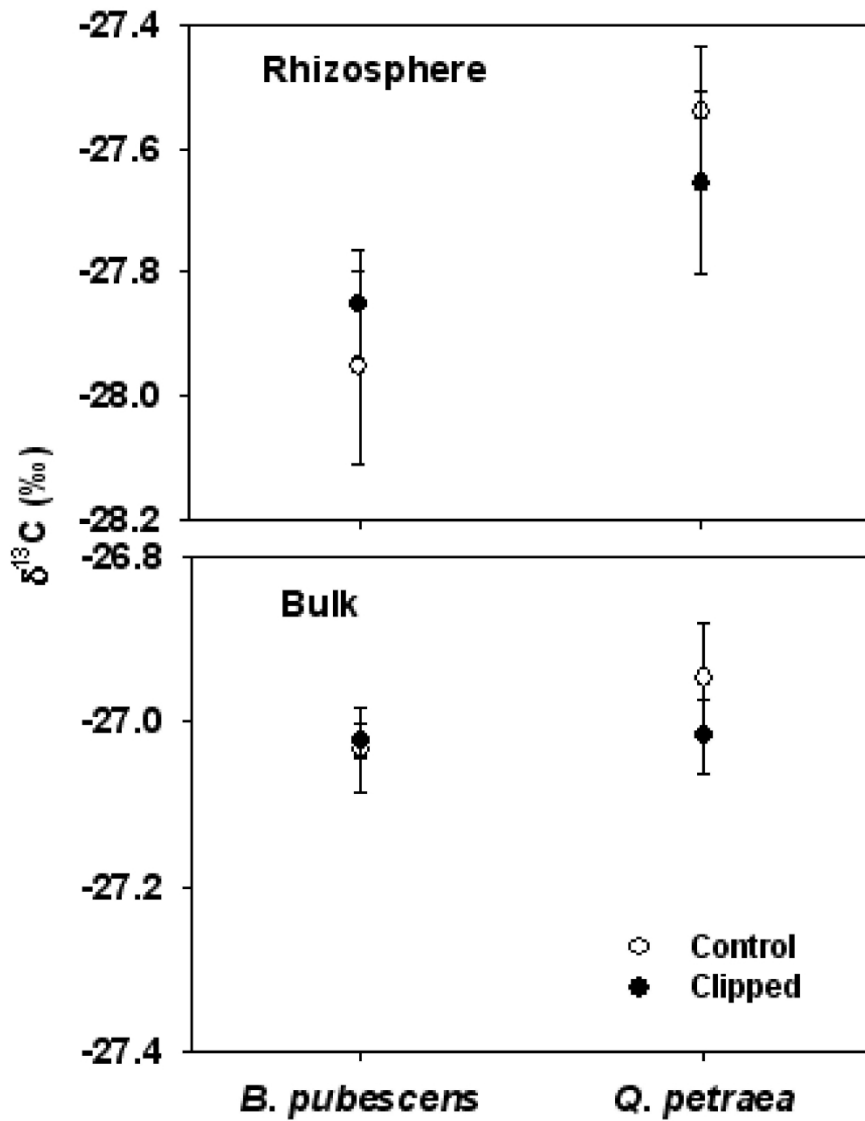


Figure 5

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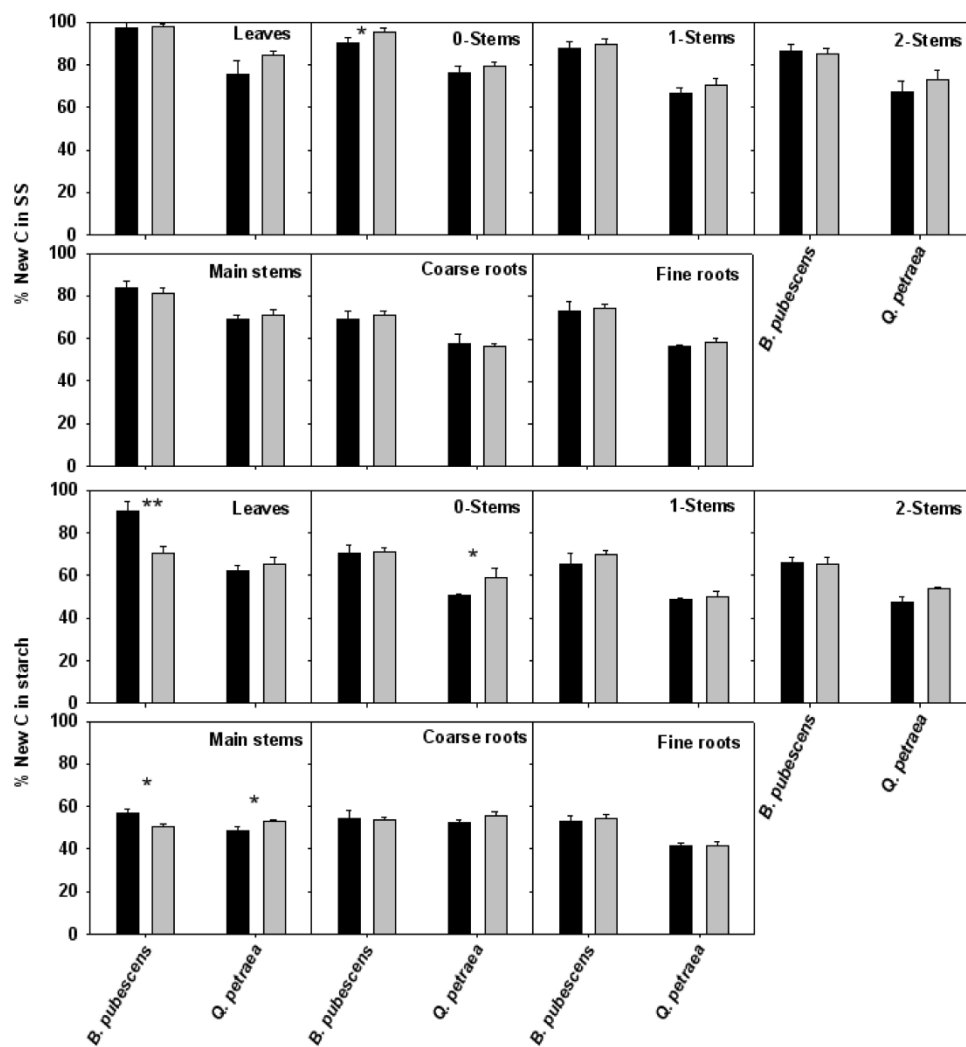


Figure 6

259x280mm (600 x 600 DPI)