1 **Browsed *Betula pubescens* trees are not carbon-limited**

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10 Running headline: C limitation in browsed trees

Summary

1. Slower growth after repetitive browsing of young trees has been attributed to a carbon (C) limitation, but data from long term studies are lacking. To determine if repeated summer browsing causes a C (source) limitation in trees in the long-term, we analysed the non-structural carbohydrate (NSC) and nitrogen (N) concentrations and pools of *Betula pubescens* saplings subjected to different clipping treatments (unclipped, 33% and 66% shoot removal) for seven years.
2. The short-term effect of browsing on C allocation was assessed by clipping trees with different browsing histories and analyzing their C and N responses within the same growing season.
3. Long-term repetitive summer browsing significantly decreased sapling growth and soluble sugar (SS) concentrations in fine roots. However, trees subjected to different browsing histories did not change their C partitioning into starch. Although trees with a different browsing history showed differences in the NSC concentrations and pools of leaves, these differences were not maintained through time.
4. Simulated browsing had no significant short-term effect on root C and N pools of birch trees, but it decreased all C pools in leaves, one-year and current-year stems and the starch and total NSC pools in main stems.
5. We conclude that although browsing may cause a temporary decrease in C pools, *B. pubescens* trees are able to compensate for such losses and hence are not C-limited in the long-term.

Key words: clipping, non-structural carbohydrates, *Betula pubescens*, herbivory, storage, nitrogen, downy birch.

1 **Introduction**

2 Browsing is an important factor inhibiting the regeneration of native woodlands in
3 many parts of the world (Hester *et al.* 2000, Côté *et al.* 2004, Gill 2006). One of the
4 direct consequences of browsing during late spring or summer is a decrease in total
5 leaf area, leading to a reduction in photosynthetic carbon (C) assimilation. Therefore,
6 it has been suggested several times that a C limitation is the cause of reduced tree
7 growth after browsing (Ericsson, Larsson & Tenow 1980, Hoogesteger & Karlsson
8 1992, Crête & Doucet 1998), including a C limitation to ectomycorrhizal symbiosis
9 (Markkola *et al.* 2004).

10 Resource use by trees has often been considered in relation to their
11 environment using C as the basic currency (Millard, Sommerkorn & Grelet 2007).
12 Implicit in this approach is the assumption that, as C₃ photosynthesis is limited by the
13 availability of CO₂, plant functioning should be considered in relation to C ‘costs’.
14 Tree growth and C status can be viewed as being either source (C acquisition) or sink
15 (C investment in growth) limited. One approach to studying this has been to consider
16 growth in relation to non-structural carbohydrates (NSC), such as starch and soluble
17 sugars, or lipid pools. Together, these represent carbon reserves or storage (*sensu*
18 Chapin, Schulze & Mooney, 1990), or possibly also sequestration if they are not
19 reusable by the tree (Millard *et al.* 2007). The idea is that a decrease in NSC pools
20 following periods of growth would provide evidence for reuse of stored C, thereby
21 demonstrating a C source (photosynthetic) limitation to growth. This approach has
22 been used to study tree growth in a wide range of ecosystems (Körner, 2003),
23 including climatic tree-lines (Hoch & Körner 2003, 2005, Shi, Körner & Hoch 2006)
24 where a temperature limitation to photosynthesis causing a C source limitation has
25 been postulated as one mechanism for tree-line formation (Stevens & Fox 1991). In

each case, with the exception of Mediterranean sclerophyllous woodlands during periods of drought, NSC pools were found to vary little either seasonally (Körner, 2003) or with altitude (Hoch & Körner, 2003; 2005; Shi *et al.*, 2006) and to be maximum during periods of reduced or zero growth. This has been interpreted as a sink limitation to growth (due to environmental or developmental constraints), rather than a limitation on C availability. Indeed, temperate, deciduous trees have been reported to contain enough NSC to replace their entire canopy four times (Hoch, Richter & Körner, 2003).

Several studies have reported a decrease in starch pools after browsing or defoliation in deciduous (Wargo, Parker & Houston 1972, Kolb *et al.* 1992, Canham, Mcaninch & Wood 1994, Van Der Heyden & Stock 1996) and evergreen species (Webb & Karchesy 1977, Ericsson *et al.* 1985, Li, Hoch & Körner, 2002). These results suggest that browsing can cause a C source (photosynthetic) limitation to the regeneration of some species. However, trees can also show compensatory increases in photosynthesis following defoliation (Hoogesteger *et al.* 1992, Lovett & Tobiessen 1993, Vanderklein & Reich 1999), explaining why in some experiments defoliation had no (or only transient) effects on NSC levels (Raitio, Paukkonen & Kauppi 1994, Tschaplinski & Blake 1994, Kosola *et al.* 2001, Handa, Körner & Hättenschwiler 2005). Despite these contrasting results, no long-term studies have been conducted on the effect of browsing on C allocation in trees. Therefore, we do not know if repetitive long-term browsing (as frequently happens in natural ecosystems) can cause a C source limitation in trees.

Betula pubescens Ehrh. (downy birch) is an early successional forest species, abundant in boreal and temperate woodlands across Europe and eastwards into Siberia (Atkinson 1992). Grazing by deer and sheep often prevents regeneration of upland

1 birch woodland (Atkinson 1992). Single browsing events have been shown to have no
2 effect on birch sapling N uptake or internal cycling (Millard *et al.* 2001, Millett *et al.*
3 2005), suggesting no N limitation to regrowth. The main NSC in *B. pubescens* is
4 starch (Mäenpää *et al.* 2001) and although several studies have assessed the seasonal
5 dynamics of NSC concentration in birches (Abod & Webster 1991, Johansson 1993,
6 Mäenpää *et al.* 2001, Piispanen & Saranpää 2001), there is little information available
7 on the NSC pools present in their different organs or how they respond to browsing.
8 The objectives of this study were to analyse both the long-term effect of repetitive
9 summer browsing and the short-term response to a single browsing event on the
10 potential for a C source or sink limitation on growth of *B. pubescens*, by an analysis
11 of the NSC pools in relation to tree size. The following hypotheses were tested, that:
12 (i) repeated summer browsing would cause a C source (photosynthetic) limitation in
13 trees, thereby leading to decreased C partitioning to NSC pools and (ii) the NSC pools
14 of trees subjected to a more intense summer browsing regime would be smaller and
15 more severely affected by short-term C losses associated with a single browsing
16 event.

17

1 **Materials and methods**

2 STUDY SITE

3 The two hypotheses of this study were assessed by two different experiments
4 conducted at the RSPB Reserve at Corrimony in north-west Scotland (57°19'N
5 4°43'W) as part of an ongoing, larger experiment set up in August 2000. The site
6 selected for the experiment contained large numbers of regenerating birch (*Betula*
7 *pubescens* Ehrh.) saplings less than 1m in height, within heather-dominated
8 vegetation. The simulated browsing (clipping) experiment set up in 2000 focused on
9 saplings approx. 30-50cm high (< 10cm below and < 15cm above heather canopy
10 height), as this was considered to be one of the most vulnerable stages of a sapling in
11 relation to large herbivore browsing (Hester *et al.* 1996, 2000, Gill 2006).

12

13 EXPERIMENTAL DESIGN

14 Three replicate plots (<20 x 20m; exact size depended on sapling density) were
15 established in August 2000 and surrounded by a stock and rabbit fence in order to
16 protect the saplings from any herbivore damage. A total of 60 saplings within each
17 plot, fitting the above size requirements in relation to the surrounding canopy, were
18 labelled and numbered (Fig. 1). Within each fenced plot, treatments were allocated to
19 randomised blocks of 5 saplings (4 blocks per treatment). Clipping treatments were
20 applied every late summer (August) over seven years, and comprised different
21 severities of clipping: control (unclipped), 33% of shoots clipped (1 out of every 3
22 shoots); 66% of shoots clipped (2 out of 3). Late summer has been suggested as the
23 time of the year when browsing is more detrimental to the general growth of
24 broadleaved tree species (Canham *et al.* 1994; Honkanen *et al.* 1994). Tree
25 morphological measurements were taken each year at the end of the growing season;

the three measurements used to characterise sapling variability for the experiments reported here were: sapling height, stem diameter (at approx 1cm above ground surface) and canopy area, measured at the start of experiment (2000) and in 2007.

Clipping treatments were designed to mimic as closely as possible browsing damage by red deer or sheep, i.e. removal of current-year long-shoots (including buds/leaves) up to the maximum stem diameters normally eaten by red deer/sheep (Shipley *et al.* 1999). As the basal diameters of all current-year shoots were within the ranges normally eaten, whole shoots were clipped. If more than one current-year shoot was present on a sapling, every second shoot was clipped to just above the point where current-year growth was initiated, starting with the leader/top shoot. When only one current-year shoot was present, one third (33% clipping treatment) or half of its length (66% clipping treatment) was removed.

In June 2007 three groups of saplings were selected from the marked individuals in each plot for each treatment (Fig. 1). The groups were selected to cover the whole size range of the study population and comprised three individuals of similar size, giving 27 saplings per plot. One sapling of each group was assigned to “Experiment 1 (long-term impacts)” and the other two were randomly allocated to the “control” and “clipping” treatments of “Experiment 2 (short-term impacts)”.

EXPERIMENT 1: LONG-TERM RESPONSES TO REPETITIVE BROWSING

To address the first hypothesis, one individual of each group or size category (9 individuals per treatment) was harvested in late June 2007, when shoot growth of *B. pubescens* had almost finished and carbohydrate storage levels have been reported to be at a minimum (Mäenpää *et al.* 2001). Whole saplings were harvested, trying to recover as many roots as possible without disturbing the rest of saplings in the plot.

Samples were taken to the laboratory immediately after harvest and stored at -20 °C, until they were separated into: leaves, current-year, one and two-year-old stems, woody stems (older than two year), coarse roots (> 2mm) and fine roots (< 2mm). Samples were freeze-dried, weighed to the closest 0.01 mg and milled to a fine powder in a ball mill (Retsch Mixer MM301, Leeds, UK).

EXPERIMENT 2: SHORT-TERM RESPONSES

The second hypothesis of the study was addressed by removing 66% of the shoots in late June 2007 from 27 of the remaining individuals (3 per plot and treatment; Fig. 1). The clippings from each sapling were kept for morphological and chemical analyses. The remaining 27 saplings (3 per plot and treatment) were left unclipped as controls (Fig. 1). Plants were left to grow for eight weeks and whole individuals were harvested as above in late August, before the onset of leaf shedding. Samples were taken to the laboratory immediately after harvest and processed as explained above for biomass and chemical analyses.

ROOT BIOMASS ESTIMATES

Due to practical reasons (disturbance of other experimental saplings), it was impossible to excavate the complete root system of all the individuals harvested in both the experiments. Consequently, the possible effect of clipping on root biomass allocation could not be accounted for in this study. The biomass and C and N pools in the main roots (> 2mm diameter) of sampled individuals were estimated by linear regression. Nine additional individual saplings growing outside the plots were fully excavated. Most roots, except very fine ones (< 2mm), were followed until their end and collected. Although these plants were not included in the fenced plots, they were

1 unlikely to have suffered from much browsing, as the deer numbers on the Corrimony
2 RSPB Reserve are kept very low to ensure native forest regeneration. Individuals
3 were selected to cover the size range of the saplings included in the experiments. The
4 excavated plants were taken to the laboratory and processed as explained above.
5 Measurements of the biomass of the different organs of these plants were used to
6 obtain the following equation relating the above and below ground biomass of *B.*
7 *pubescens* saplings by linear regression (Genstat 9):

8
$$\text{Log}(CR) = 0.985 * \text{Log}(AW) - 0.124 \quad (R^2 = 0.92, P < 0.001) \quad [\text{Eq. 1}]$$

9 Where CR = coarse roots biomass and AW = aboveground woody biomass. This
10 equation was subsequently used to estimate the coarse root biomass of the saplings
11 harvested within the experimental plots.

12

13 CHEMICAL ANALYSES

14 Nitrogen (N) mass-based concentrations were measured with an elemental analyzer
15 (Elementar VarioMAX N/CM, Hanau, Germany). Soluble sugars were extracted with
16 80% (v/v) ethanol and their concentration determined colorimetrically, using the
17 phenol-sulphuric method of Dubois *et al.* (1956) as modified by Buysse & Merckx
18 (1993). Starch and complex sugars remaining in the undissolved pellet after ethanol
19 extractions were enzymatically reduced to glucose and analyzed as described in
20 Palacio *et al.* (2007). Non-structural carbohydrates measured after ethanol extraction
21 are referred to as soluble sugars (SS) and carbohydrates measured after enzymatic
22 digestion in glucose equivalents are referred to as starch.

23

24 CALCULATIONS AND STATISTICS

1 The first step in our analysis was to identify the main C storage pools in *B. pubescens*.
2 Information on the amount of NSC, i.e. the pools, rather than just mass-based
3 concentrations, is needed to understand changes in C storage in response to
4 disturbances (James, 1984). Trees may have similar NSC concentration but if the size
5 of their storage organs differs, their NSC pools and hence the potential ability to
6 survive disturbances will be different (Iwasa & Kubo 1997). Starch, SS, NSC and N
7 pools were calculated by multiplying the mass-based concentrations of the different
8 organs of unclipped trees (from Experiment 1) by their biomass. Coarse roots biomass
9 was estimated by using the equation described above [Eq. 1]. Differences in the
10 biomass and N, SS, starch and NSC pools and concentrations of the different fractions
11 among clipping treatments were assessed by residual maximum likelihood (REML,
12 Genstat 9) analysis, with “long-term clipping” as a fixed factor, “plot” as a random
13 factor and the logarithm of aboveground woody biomass at harvest as a covariate. The
14 use of covariates in REML analyses allowed explicit examination of the variation
15 between individuals caused by previous differences in the size and morphology of
16 individuals, and not by treatments. Log-transformed values were used for pools in all
17 comparisons to meet the assumptions of REML. Differences between individual
18 means were tested using least significant differences (LSDs) calculated directly from
19 the REML analysis.

20 In order to study if repeated summer clipping over seven years had had any
21 effect on the growth (height, canopy area and stem diameter at the end of growth
22 season) of birch saplings, REML analysis was used with long-term clipping as fixed
23 factor, plot as a random factor, and pre-treatment height and stem diameter measured
24 in August 2000 as covariates. All variables were log-transformed to meet the

requirements of REML. Differences between individual means were tested using LSDs calculated directly from the REML analysis.

Because pools are inherently affected by tree size, the effect of repetitive summer browsing on the C partitioning into starch was assessed by non-linear regression analysis between starch pools ($\text{g starch plant}^{-1}$) and the logarithm of the total biomass of the trees (Experiment 1). In this way, differences in C partitioning into starch pools could be assessed independently of tree size. Separate curves were fitted to the different long-term clipping treatments (33%, 66% and control) and compared to assess if clipping modifies the amount of C partitioned to starch per g of plant biomass. A C (source) limitation in trees due to browsing would be seen as a decrease in the partitioning of C into starch, as clipping intensity increases. Accordingly, the slope of the curve of the 66% treatment would be the lowest, and than that of the control the largest.

REML analyses were also used to assess the short-term effect of summer browsing on trees previously subjected to different long-term clipping treatments (Experiment 2). The model comprised: short-term clipping (66% clipped and control) and long-term clipping treatment (33%, 66% and control) as fixed factors; plot as a random factor; and the logarithm of aboveground woody biomass at harvest as a covariate. Pool values were log-transformed to meet the requirements of REML.

Results

DISTRIBUTION OF STARCH AND SS POOLS

Coarse roots and main stems contained the majority of the NSC pools in *B. pubescens*, accounting for 87- 94% of the total starch and 70-87 % of the total SS pool of trees (Fig. 2). The amount of NSC accumulated in young twigs, i.e. current-year, one and two year old stems, was very low, and even when combined together in one fraction they accounted for only 1.5% of the total pool of birch saplings (Fig. 2).

EFFECT OF REPETITIVE CLIPPING ON GROWTH

Long-term repeated summer clipping resulted in significantly smaller saplings (Table 1). The height, canopy area and stem diameter of control trees was always larger than 33% clipped trees, which in turn were larger than 66% trees (Table 1). Although trees from different treatments were similar in size at the beginning of the study (2000), differences between treatments ($P < 0.001$) were highly significant after seven years of repetitive clipping.

EFFECT OF REPETITIVE CLIPPING ON NSC POOLS

The relationship between the amount of starch present in the main woody organs (coarse roots and main stems) and total plant biomass was highly significant ($P < 0.001$) and explained 95.5 % of the total variability of the data (Fig. 3). However, contrary to our first hypothesis, the curves fitted to the three long-term clipping treatments were not significantly different from each other ($P = 0.982$), and hence there was no treatment effect on the C partitioning to starch.

The comparison of the average C and N pools of the different organs by REML gave similar results. Leaf NSC pools in trees subjected to 66% clipping were significantly lower than control and 33% trees in Experiment 1 (Table 2). However, these differences could not be found in trees harvested for Experiment 2 in late August (Table 3). The only significant effect of long-term browsing that was consistent through time was a decrease in SS concentrations in fine roots ($P = 0.035$). Apart from this, there were no significant differences in the C and N pools or concentrations in the different organs of trees, irrespective of their treatment (Tables 2 and 3).

SHORT-TERM EFFECT OF CLIPPING

Short-term responses to clipping were assessed by measuring C and N pools in trees harvested in Experiment 2, eight weeks after clipping. There were no significant short-term effects on the SS, starch, total NSC or N concentrations or pool sizes in the coarse roots, fine roots and two-year-old twigs of *B. pubescens* (Table 3; Figs. 4 and 5). However, clipping decreased the starch and total NSC concentration and pools in main stems ($P < 0.05$), all the C pools in leaves, current and one-year-old stems and the N pools of leaves and current-year stems (Table 3; Figs. 4 and 5).

Trees subjected to different long-term clipping treatments showed no significant differences in their short-term response to clipping. The only significant differences in the interaction between short and long-term clipping were found in the starch concentration of leaves ($P = 0.036$) and the total NSC concentration of two-year-old stems ($P = 0.004$).

1

2 **Discussion**

3 Many previous studies have found that browsing or defoliation causes a decline in
4 starch reserves (Wargo *et al.* 1972, Webb *et al.* 1977, Ericsson *et al.* 1985, Ericsson *et*
5 *al.* 1980, Kolb *et al.* 1992, Canham *et al.* 1994, Li *et al.* 2002, Van Der Heyden *et al.*
6 1996). Our results agree with these previous studies in the short- but not in the long-
7 term, as annual clipping for seven years had no effect on the C partitioning to starch.
8 Consequently, although tree growth was reduced by simulated long-term browsing, it
9 was not due to a C source (photosynthetic) limitation.

10 Three factors can explain these contrasting results. First, in many of the
11 studies that found a significant reduction of C reserves, this was caused only by
12 complete or very heavy defoliation (removing more than 90% of the leaves), while the
13 effect of less harsh treatments were not significant (c.f. Kolb *et al.* 1992, Tschaplinski
14 *et al.* 1994, Van Der Heyden *et al.* 1996, Wargo *et al.* 1972). Natural browsing, even
15 if repeated over time, does not normally result in complete defoliation of trees (Gill
16 1992). Compensatory increases in the photosynthesis of surviving leaves
17 (Hoogesteger *et al.* 1992, Lovett *et al.* 1993) may suffice to compensate for the C
18 losses associated with browsing or light defoliation experiments (Tschaplinski *et al.*
19 1994). For example, Van der Heyden & Stock (1996), demonstrated that plants of the
20 deciduous shrub *Osteospermum sinuatum* were able to over-compensate NSC pools in
21 twigs just 4 weeks after clipping, despite twig biomass having been reduced by 80%.

22 Secondly, in many studies the decrease in C pools following clipping was only
23 transient (Kosola *et al.*, 2001), or only short-term effects were assessed (c.f. Buwai &
24 Trlica 1977, Canham *et al.* 1994, Li *et al.* 2002, Wargo *et al.* 1972). In our study,
25 simulated browsing had a significant short-term impact on the NSC pools of main

1 stems, and decreased the N and C pools of all the fractions directly affected by
2 clipping (such as leaves and young twigs). However, trees were able to compensate in
3 the longer term, so that there were no differences between treatments in the NSC
4 pools of stems or coarse roots. On the other hand, the differences we found in the
5 NSC pools of leaves of different long-term clipping treatments were not consistent
6 through time, which indicates that they were merely due to changes in phenology.
7 Previous studies have shown that browsing can modify bud burst dates in some
8 species (Hester *et al.* 2004), which may have a subsequent impact on the timing of
9 other phenological stages. Such phenological differences can lead to variations in
10 NSC and N seasonal dynamics (Milla *et al.* 2005; Palacio *et al.* 2007), which would
11 explain the contrasting results obtained for different sampling dates in this study.

12 Finally, many of the previous studies that found a significant decrease in C
13 stores after browsing were conducted on evergreen conifers (Ericsson *et al.* 1980,
14 1985, Li *et al.* 2002, Webb *et al.* 1977) which, in contrast to winter-deciduous trees,
15 store their C reserves in leaves (Kozlowski & Keller 1966). For example needles
16 accounted for up to 68% of the total C pool of *Pinus cembra* trees, while roots
17 accounted only for 17% (Li *et al.* 2002). These numbers are very different to the ones
18 reported in this study for *B. pubescens*, or those cited in the literature for other winter
19 deciduous species, where coarse roots and main stems account for most of the NSC
20 pools of the plant (Abod *et al.* 1991, Loescher, McCamant & Keller 1990).
21 Consequently, browsing or defoliation in evergreen conifers implies not only
22 decreasing total leaf area, as in winter deciduous species, but also eliminating most of
23 the C (Li *et al.* 2002) and N (Millard *et al.* 2001) stores of the plant. It is, therefore,
24 not surprising that the effects of browsing or defoliation are more conspicuous in
25 evergreen conifers, where even light defoliation treatments can cause a significant

1 decrease in NSC pools (Ericsson *et al.* 1980, 1985). Nevertheless, even when severe
2 defoliation was applied, evergreen conifers did not seem to be C source limited either,
3 as their C stores were never depleted and trees were able to recover most of the initial
4 decrease by the end of the first growing season (Ericsson *et al.* 1985, Li *et al.* 2002,
5 Vanderklein *et al.* 1999).

6 Repeated summer browsing did not cause a C source limitation in *B.*
7 *pubescens* trees, but it did markedly decrease their growth. What factors other than C
8 availability could account for such reduced growth? Repetitive defoliation has been
9 suggested to cause a nutrient limitation in tree growth by decreasing tree N content,
10 increasing fine root mortality and subsequently reducing nutrient uptake (Tuomi,
11 Niemelä & Sirén 1990, Bryant *et al.* 1993). However, differences in N concentrations
12 and pools among long-term browsing treatments were not significant in this study.
13 Similarly, Millard *et al.* (2001) and Millett *et al.* (2005) found no significant effect of
14 a single browsing event on the N remobilization or N uptake by birch, and Danell,
15 Haukioja & Huss-Danell (1999) found no evidence for N limitation in clipped birches
16 along a gradient of plant productivity. Therefore, the current data suggest that an N
17 limitation is unlikely, even after repeated browsing events over a number of years.

18 An alternative explanation is a C sink limitation to growth as a consequence of
19 repeated clipping, due to a cumulative effect on buds. A significant proportion of buds
20 are removed each time shoots are browsed. Birch trees show abundant basal buds
21 which originate from the branching and development of axillary buds in young
22 seedlings (Kauppi, Rinne & Ferm 1987, Stone & Cornwell 1968). These basal buds
23 normally provide sufficient number of resprouts to compensate shoot loss due to
24 browsing. However, the number of basal buds seems to be affected by seedling age
25 and vigour (Stone & Cornwell 1968). This may explain why many of the buds of

saplings subjected to a repeated 66% summer clipping (the least vigorous ones) were dead and resprouts were scarce (data not shown). Other studies of NSC pools in trees have also concluded that growth in a wide range of ecosystems is C sink, not source limited (Körner 2003, Hoch & Körner 2003, 2005, Shi *et al.* 2006).

To conclude, this work demonstrates that summer clipping can decrease C pools in the short but not in the long-term. Trees are apparently able to compensate for C losses derived from browsing, so that repetitive summer clipping performed over seven years does not change the C partitioning to NSC pools of *B. pubescens* trees growing in the wild. Differences with previous studies can be explained by differences in treatment intensity, time elapsed after the application of treatments and the species involved in the study. Altogether, our results support the view that trees are not C (source) limited.

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1 **Figure legends**

2 **Fig. 1.** Diagram showing the lay-out of the different experiments included in this
3 study.

4 **Fig. 2.** Percentage of the total starch and soluble sugars (SS) pool accounted for by the
5 leaves (white bars), young twigs (dashed bars), main stems (grey bars) and coarse
6 roots (black bars) of an average *Betula pubescens* tree. Values are means (n = 9).

7 **Fig. 3.** Relationship between starch pool in the coarse roots and main stems of *B.*
8 *pubescens* (g plant⁻¹) and the logarithm of the total plant biomass. Different symbols
9 indicate different long-term clipping treatments: control (●); 33% of current year's
10 shoots removed (○); and 66% of current year's shoots removed (▼). Data points were
11 fitted to an exponential curve with the equation: $y = -2.70 + 0.0233 (3.566^x)$; $R^2 =$
12 0.95, $P < 0.001$.

13 **Fig. 4.** Soluble sugars (white bars) and starch (grey bars) concentrations (mg g⁻¹) in
14 the different organs of *B. pubescens* trees subjected to different long-term (33%, 66%
15 and unclipped control) and short-term (B = 66% of current year's shoots removed in
16 June 2007, C = unclipped) clipping treatments. Vertical lines indicate LSDs of starch
17 (upper line) and SS (lower line) concentration. Values are means (n = 9).

18 **Fig. 5.** Starch, soluble sugars (SS), total non-structural carbohydrates (NSC) and
19 nitrogen (N) pools in the leaves (white bars), young twigs (dashed bars), main stems
20 (grey bars) and coarse roots (black bars) of *B. pubescens* trees subjected to different
21 long-term (33%, 66% and unclipped control) and short-term (B = 66% of current
22 year's shoots removed in June 2007, C = unclipped) clipping treatments. Values are
23 means (n = 9). Log-transformed LSDs for starch: coarse roots = 0.2273, main stems =
24 0.1323, young twigs = 0.4350, leaves = 0.4138. Log-transformed LSDs for SS: coarse
25 roots = 0.1212, main stems = 0.0998, young twigs = 0.3942, leaves = 0.4257. Log-

- 1 transformed LSDs for NSC: coarse roots = 0.1609, main stems = 0.0878, young twigs
- 2 = 0.4059, leaves = 0.4117. Log-transformed LSDs for N: coarse roots = 0.1127, main
- 3 stems = 0.1092, young twigs = 0.4768, leaves = 0.3816.

4

1 **Table 1.** Height, canopy area and stem diameter in the three long-term clipping
2 treatments (33%, 66% and unclipped control) at the start (2000) and the end (2007) of
3 the study.

	Height (m)	Canopy area (m ²)	Stem diameter (mm)
2000			
<i>Control</i>	0.40 ± 0.07 ^a	0.02 ± 0.02 ^a	5.1 ± 1.37 ^a
33%	0.39 ± 0.08 ^a	0.02 ± 0.02 ^a	5.1 ± 1.45 ^a
66%	0.40 ± 0.08 ^a	0.02 ± 0.01 ^a	4.5 ± 1.20 ^a
2007			
<i>Control</i>	0.81 ± 0.21 ^a	0.14 ± 0.11 ^a	12.0 ± 4.42 ^a
33%	0.62 ± 0.30 ^b	0.09 ± 0.11 ^b	8.9 ± 4.36 ^b
66%	0.45 ± 0.20 ^c	0.02 ± 0.02 ^c	6.2 ± 1.77 ^c

4 Values are means ± SD. N = 60. Different letters indicate significant differences after
5 LSDs calculated directly from REML analyses.

6

Table 2. Summary of statistics for Experiment 1 (long-term effects) in June 2007. Differences between treatments in starch, soluble sugars (SS), total non-structural carbohydrate (NSC) and nitrogen (N) pools were assessed by using residual maximum likelihood (REML, see materials and methods for further details). The model comprised long-term clipping treatment (33%, 66% and unclipped control) as a fixed factor; plot as a random factor and the logarithm of the total woody biomass at the time of harvest as a covariate.

	Starch		SS		NSC		N	
	Wald	P	Wald	P	Wald	P	Wald	P
<i>Coarse Roots</i>	4.19	0.151	1.24	0.550	2.83	0.267	5.28	0.104
<i>Main Stems</i>	3.99	0.165	0.39	0.824	4.30	0.146	0.39	0.824
<i>Leaves</i>	10.89	0.014	9.80	0.019	10.49	0.015	1.51	0.498
<i>Current-year stems</i>	1.36	0.539	1.06	0.615	1.11	0.599	-	-
<i>One-year stems</i>	2.15	0.388	0.40	0.831	1.14	0.593	-	-
<i>Two-year stems</i>	4.04	0.184	0.87	0.658	2.21	0.369	0.05	0.842

Wald statistic and P-values are shown. Significant differences between treatments are indicated in bold. D. f. = 2.

Table 3 Summary of statistics for Experiment 2 (short- and long- term effects) in August 2007. Differences between treatments in starch, soluble sugars (SS), total non-structural carbohydrate (NSC) and nitrogen (N) pools were assessed by using REML. The model comprised long- (33%, 66%, control) and short-term clipping (66%, control) as fixed factors; plot as a random factor and the logarithm of the total woody biomass at the time of harvest as a covariate.

	Starch		SS		NSC		N	
	Wald	P	Wald	P	Wald	P	Wald	P
Long-term effect								
<i>Coarse Roots</i>	0.60	0.742	5.81	0.067	0.33	0.847	10.62	0.009
<i>Main Stems</i>	0.63	0.732	< 0.01	0.999	0.76	0.686	0.74	0.692
<i>Leaves</i>	1.28	0.532	1.55	0.468	1.26	0.538	0.40	0.821
<i>Current-year stems</i>	0.06	0.969	0.01	0.994	0.05	0.977	0.93	0.636
<i>One-year stems</i>	5.04	0.097	7.51	0.035	6.36	0.555	10.77	0.013
<i>Two-year stems</i>	0.28	0.871	1.14	0.573	0.60	0.742	2.03	0.377
Short-term effect								
<i>Coarse Roots</i>	0.03	0.867	1.75	0.193	< 0.01	0.958	1.40	0.243
<i>Main Stems</i>	8.26	0.006	0.60	0.442	11.36	0.002	0.22	0.644
<i>Leaves</i>	13.24	< 0.001	12.67	< 0.001	13.48	< 0.001	11.26	0.002
<i>Current-year stems</i>	17.57	< 0.001	15.84	< 0.001	16.96	< 0.001	34.87	< 0.001
<i>One-year stems</i>	5.34	0.028	5.48	0.026	5.77	0.023	2.88	0.104
<i>Two-year stems</i>	1.91	0.177	1.40	0.245	1.83	0.186	0.26	0.614

Wald statistic and P-values are shown. Significant differences between treatments are indicated in bold. D. f. = 2 for long-term treatment and D. f. = 1 for short-term treatment effects.

1 **Fig. 1.**

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1 **Fig. 2.**

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1 **Fig. 3.**

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1 **Fig. 4.**

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1 **Fig. 5.**

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