Foliar habit, tolerance to defoliation and their link to carbon and nitrogen storage

Frida I. Piper* and Alex Fajardo

Centro de Investigación en Ecosistemas de la Patagonia (CIEP) Conicyt–Regional R10C1003, Universidad Austral de Chile, Camino Baguales s/n, Coyhaique, Chile

Summary

1. An innovative hypothesis to explain the higher carbon (C) and nitrogen (N) storage in woody tissues of winter deciduous species as compared to evergreen species is that these storages reflect an adaptation to tolerate herbivory. Support for this hypothesis has been little when manipulative defoliations were partial and/or applied in a single season. Given that repeated defoliations throughout a single season are common in temperate forests and tend to be more severe in deciduous than in evergreen species, we tested this hypothesis considering complete and recurrent defoliation in two sympatric *Nothofagus* species with contrasting foliar habit.

2. In the field, we applied three defoliation intensities for 3 years in naturally coexisting juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous). Defoliation intensities included complete defoliation (100%) twice during the growing season, partial defoliation (50%) twice during the whole experiment and no defoliation. We evaluated survival, regrowth and C- and N-storage in the leaves, stems and roots of each tree.

3. Complete defoliation caused 100% mortality in *N. betuloides* after the first year and no mortality in *N. pumilio* after 3 years; it induced higher C reductions in *N. pumilio* roots, supported by greater C-storage. Partial defoliation caused no interspecific differences in survival, though it produced a stronger decrease in C-storage in *N. betuloides* than in *N. pumilio*. N concentrations in woody tissues were significantly higher in *N. pumilio* than in *N. betuloides*, and only in the former did they decrease with the defoliation intensity.

4. *Synthesis*. We found a potential functional link between leaf habit, defoliation tolerance and C- and N-storage. The deciduous species tolerated complete and recurrent defoliations better than the evergreen species, which was associated with higher C- and N-storage in stems and roots of the former. This link was not detected under partial defoliation. We suggest that the higher C- and N-storage in the woody tissues of deciduous species as compared to evergreen species is an adaptation to tolerate complete and recurrent defoliations under which temperate winter deciduous species may have evolved.

Key-words: carbon limitation, deciduous, disturbances, ecophysiology, evergreen, herbivory, *Nothofagus betuloides*, *Nothofagus pumilio*, Patagonia, temperate forests

Introduction

Evergreen species differ from winter deciduous species in that they have functional leaves year-round. A less conspicuous but distinctive physiological trait differentiating the two leaf habits relates to carbon (C) and nitrogen (N) storage in perennial, woody tissues, which is higher in deciduous than in evergreen tree species (e.g. Vanderklein & Reich 1999; Millard *et al.* 2001; Hoch, Richter & Körner 2003; Millard & Grelet 2010; Fajardo, Piper & Hoch 2013; Richardson *et al.*

2013). The ecological and evolutionary reasons for the different C- and N-storage in these leaf habits are not clear. The classical and most accepted explanation affirms that winter deciduous species are highly dependent on C- and N-storage in order to meet spring growth demands (Dickson 1989; Kozlowski 1992; Pallardy 2007). This explanation is largely based on species artificially selected for high fruit or wood yield, which is necessarily linked to important seasonal fluctuations in C- (e.g. Boscagli 1982; Mataa & Tominaga 1998; Mataa, Tominaga & Kozaki 1998; Miller *et al.* 1998) and N-storage (Tromp 1983). In wild winter deciduous species, however, the amount of carbohydrates withdrawn from major

*Correspondence author: E-mail: fpiper@ciep.cl

C-storage sites in spring is actually a very small fraction of the total (Barbaroux & Bréda 2002; Hoch, Richter & Körner 2003; El Zein *et al.* 2011; Richardson *et al.* 2013), and variations in C-storage are restricted to the youngest shoots during short periods (Schädel *et al.* 2009), consistent with the rapid C-autonomy acquired by new leaves and shoots (Hoch, Richter & Körner 2003; Keel & Schädel 2010; Landhäusser 2011). As such, winter deciduous species show similar annual variation in C-storage when compared to evergreen species (Hoch, Richter & Körner 2003), as well as similar relative contributions of N-storage mobilization and uptake for growth demands (Millard 1994; Grelet *et al.* 2001; Silla & Escudero 2003). Thus, even during the highly demanding period of leaf flushing, woody tissues of deciduous species maintain higher storage of both C and N than those of evergreens (Millard 1994; Grelet *et al.* 2001; Hoch, Richter & Körner 2003), undermining the phenological hypothesis. Thus, an unresolved question is: why do winter deciduous species store more C and N in their woody tissues?

It has been proposed that high levels of C- and N-storage in woody tissues of winter deciduous trees may reflect an adaptation to tolerate defoliation (Millard *et al.* 2001; Hoch, Richter & Körner 2003). The idea is consistent with the higher defoliation levels that deciduous species seem to experience. The fast leaf economics strategy of deciduous species, associated with low toughness and high nutrient concentration and specific leaf area (SLA; Mooney & Gulmon 1982; Walters & Reich 1999; Wright *et al.* 2004), certainly makes leaves more susceptible to herbivory (Chabot & Hicks 1982; Pringle *et al.* 2011). Abiotic disturbances like frost, wind, severe drought and hail are other important agents of defoliation in temperate forests (Grayburn 1957; Dobbs & McMinn 1973; Ozolincius & Stakenas 1996; Lorenz & Becher 2012), which also appear to cause more defoliation in deciduous than in evergreen species (Lorenz & Becher 2012). In cases of intense and unpredictable defoliation, herbivory tolerance is thought to be the most efficient strategy to adopt (van der Meijden, Wijn & Verkaar 1988; Haukioja & Koricheva 2000). One of the main physiological mechanisms behind herbivory tolerance is the mobilization of stored C and nutrients (van der Meijden, Wijn & Verkaar 1988; Richards 1993; Tiffin 2000; Myers & Kitajima 2007; Palacio *et al.* 2008). Thus, higher C- and N-storage in deciduous species as compared to evergreen ones may be an adaptation to tolerate the defoliations that deciduous species seem to have evolved with.

The adaptive role of C- and N-storage in the herbivory tolerance of deciduous species has, however, not been supported much in previous studies. In particular, studies assessing partial defoliations have not found that winter deciduous species are more tolerant to defoliation or that they experience higher C mobilization than evergreens. For instance, Vanderklein & Reich (1999) found that seedlings' survival and C reserves of *Larix leptolepis* (deciduous) and *Pinus resinosa* (evergreen) were similar in response to 25–75% defoliation; Handa, Körner & Hättenschwiler (2005) found

that both adult trees of *Larix decidua* and *Pinus uncinata* survived a single 80% defoliation event and recovered their initial needle length and most of their leaf carbohydrate concentrations after 3 years. Similar responses to partial defoliation in both leaf habits can be explained by mechanisms such as compensatory photosynthesis (Vanderklein & Reich 1999; Li, Hoch & Körner 2002; Handa, Körner & Hättenschwiler 2005), remobilization from remaining leaves (Millard *et al.* 2001) and fast C-autonomy of new shoots and leaves (Keel & Schädel 2010; Landhäusser 2011), all of which can make the retrieval of C-storage in both leaf types unnecessary. And yet, under complete or very severe defoliations, deciduous species have a clear advantage, since, in contrast to evergreens, they store C and N in the woody tissues, not in the leaves (Millard *et al.* 2001; Millard & Grelet 2010). Although partial defoliations certainly represent the most common case for many temperate forests, complete defoliations can also be very common and more detrimental in temperate forests (Kulman 1971; Veblen *et al.* 1996; Freelich 2002; Paritsis 2009; Paritsis *et al.* 2010; F. I. Piper & A. Fajardo, pers. obs., see Fig. 1). Thus, we propose that the high C- and N-storage in deciduous species' woody tissues reflects an adaptation to tolerate complete and repeated defoliations, rather than partial ones.

In this study, we aimed to determine the defoliation tolerance of two sympatric, congeneric broadleaf tree species with contrasting leaf habits: *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous). The two species coexist along a wide latitudinal range in southern South America (approximately 40–55°SL). Previous work has demonstrated that along a temperature gradient, C-storage has always been higher in *N. pumilio* than in *N. betuloides*

Fig. 1. Stands of *Nothofagus pumilio* showing complete defoliation after a massive outbreak by the caterpillar of the moth *Ormiscodes amphimone* (Lepidoptera: Saturniidae) in southern South America (Estero Parada area in Reserva Cerro Castillo, Región de Aysén, Chilean Patagonia). The outbreak's peak was in January 2009. The picture was taken in April 2009 (mid-autumn); thus, the reddish canopy at the higher elevation corresponds to the forest which was not defoliated due to the caterpillars' thermal threshold. Note the green (i.e. new) leaves produced after the defoliation event on the tree in the front of the photograph.

(Fajardo, Piper & Hoch 2013). Here, we hypothesize that *N. pumilio* has higher tolerance to complete and recurrent defoliation than *N. betuloides*, due to higher C- and N-storage in woody tissues. Massive and complete defoliations by the caterpillar *Ormiscodes amphimone* (Lepidoptera: Saturniidae) are common in *N. pumilio* forests, and they can occur during consecutive years (Paritsis *et al.* 2010; Fig. 1). *Nothofagus betuloides* may also be affected by *O. amphimone*, but to a much lower degree than *N. pumilio* (Veblen *et al.* 1996; Paritsis 2009; Paritsis *et al.* 2010). In addition, southern South American *Nothofagus* forests have evolved under a regime of large-scale, frequent and catastrophic disturbances, where abiotic defoliations have been reported (Veblen *et al.* 1996). Using a purported, different-intensity defoliation experiment (zero, partial and complete) in juveniles of *N. betuloides* and *N. pumilio*, we examined survival, regrowth and C- and N-storage in a period of three growing seasons. Specifically, we determined whether the deciduous species had higher C- and N-storage in their woody tissues than the evergreen species. If so, we expect that: (i) under complete defoliation events, *N. pumilio* will make use of its higher C- and N-storage to promptly replace foliage tissue and eventually survive, incurring stronger C and N reductions; (ii) *N. betuloides*, on the other hand, will run out of reserves under complete defoliation and will most likely die; and (iii) when defoliation is of partial intensity, both leaf types will be able to survive, resulting in similar and generally low C or N mobilization.

Materials and methods

SPECIES AND STUDY SITE

Nothofagus betuloides (Mirb.) Oerst. and *N. pumilio* (Poepp. Et Endl.) Krasser (Nothofagaceae) are two broadleaf tree species endemic to the southern Andes of South America, distributed from 40° to 55°S and from 35° to 55°S, respectively (Rodríguez & Quezada 2003). They are among the most cold-resistant tree species of the region forming high-elevation forests (Alberdi *et al.* 1985; Fajardo, Piper & Hoch 2013). *Nothofagus betuloides* is an evergreen tree with leaf longevity of at least 2 years (A. Fajardo & F. I. Piper, unpubl. data). *Nothofagus pumilio* is a winter deciduous tree whose growing season typically starts in October and extends to mid-April, although this varies with latitude and elevation (Hevia *et al.* 1999). In Patagonia, leaf bud break occurs in late October, maximum leaf size is reached in early December, leaf senescence (reddish colour) occurs in April, and complete leaf shedding is achieved in May (A. Fajardo & F. I. Piper, unpubl. data).

The study site where the experiment was located is in the Alto Río Ibáñez area (46°10′S, 72°38′W, 550 m a.s.l.) in the Aysén Region of the Chilean Patagonia. The mean annual precipitation is 1600 mm and is distributed regularly throughout the year (Dirección General de Aguas, Servicio Meteorológico Nacional); mean temperature for the growing season is 8.6 °C. The forest here is dominated by *N. betuloides* and *N. pumilio*. We worked in an open-canopy mature forest site (< 150 trees ha^{-1}, 20–25 m high), where juveniles (1–1.5 m high) of both species co-occur intermingled at a high density (approximately 2500 juveniles ha^{-1}).

DEFOLIATION EXPERIMENTAL DESIGN

Within the forest, we set 10 sampling points every 25 m along a sampling line. At each sampling point we selected in the neighbourhood three juvenile trees of *N. pumilio* and *N. betuloides* (1.5 m high, approximately 10 years old). Juveniles' selection was based on the following criteria: unshaded and vigorous looking, one dominant stem, no signs of disease or herbivory and no close (< 50 cm) neighbours (i.e. minimum biotic interactions). Three levels of defoliation were randomly assigned to juveniles of each species: 100%, 50% and 0% of defoliation (i.e. control). Thus, we had six treatments (two species × three defoliation levels) with 10 replicates each. One hundred percent defoliation consisted of the complete removal of leaves both in mid-December (late spring) 2009, 2010 and 2011, and in early February (mid-summer) 2010 and 2011. Fifty percent defoliation consisted of the removal of every other leaf in late spring twice during the whole experiment (mid-December 2009 and 2010). When we carried out subsequent defoliations, we made no distinctions between leaves displayed before and after defoliation, that is, all leaves were treated equally disregarding leaf age. Defoliations were performed manually, and we were particularly careful to not remove buds. Mean leaf size was 1.73 cm^2 (±0.2) for *N. betuloides* and 2.6 cm^2 (±0.6) for *N. pumilio*. For complete defoliation, we defoliated on average 2135 (±220) leaves for each *N. betuloides* juvenile and 1068 (±146) leaves for each *N. pumilio* juvenile.

SURVIVAL AND REGROWTH

At each defoliation date, we evaluated survival and regrowth (i.e. leaf biomass produced following a defoliation event). Juveniles were considered dead when all their leaves, buds and bark (slightly removed at the base of the stem with a knife) were brown and dry. For 100% defoliation, regrowth was determined as the percentage of dry leaf mass and leaf area harvested at each defoliation date relative to the initial dry leaf mass and leaf area, respectively. While less straightforward, for 50% defoliation, regrowth was estimated 1 year after the beginning of the experiment and at the end, with the following formula:

$$R = (l_h * 2 - l_r) * 100/l_i$$

where R stands for regrowth (in percentage), l_h is the harvested leaf biomass at a given date (i.e. l_h*2 represents the total leaf biomass at a given date), l_r is the leaf biomass harvested at the previous defoliation date and l_i is the initial leaf biomass (estimated as twice the harvested leaf biomass at the first defoliation date; Fig. S1 in Supporting Information). Leaf biomass for l_h, l_r and l_i was measured in grams of dry weight. For this, harvested leaves from each treatment and sampling date were collected, placed in labelled bags and stored in cool boxes that were brought to the laboratory (Centro de Investigación en Ecosistemas de la Patagonia, Coyhaique). Given that regrowth was based on dry leaf mass and leaf area, we calculated SLA, determined as the ratio between fresh leaf area and leaf mass. For leaf area determination, a subset of fresh leaves from each juvenile was displayed separately on a white sheet along with a reference square of known size (4 cm^2), covered with a transparent glass and photographed with a Sony DSC–S750 digital camera (Sony Corporation, Tokyo, Japan). Images were later processed to calculate the total projected leaf area using the image processing software SIGMAPROC 5.0 (Systat Software Inc., Richmond, CA, USA). Leaves were then placed to dry in a forced-air stove (Memmert GmbH, Schwabach, Germany) at 70 °C for 72 h and were subsequently weighed with a scale to a precision

of 0.0001 g. Finally, the successive measurements of regrowth were added for each juvenile to estimate the cumulative regrowth.

HARVEST AND BIOMASS ANALYSES

Trees were sampled for chemical and biomass analyses at the end of the experiment in March 2012 (late summer). As an exception, *N. betuloides* juveniles which had undergone 100% defoliation were found dead in mid-December 2010 (late spring) and were thus harvested for chemical and biomass analyses at that time. A few brownish and dried leaves, still attached to the dead juveniles, were used for analyses. We do not know exactly when these juveniles died; however, they probably had not yet begun to decompose at the harvest time, and we therefore assume our data are indicative of the real physiological condition of the juveniles at their time of death. We believe that the low temperatures which occur in the winter and spring in this geographical area most likely impede fast tissue decomposition. Nevertheless, to test for potential changes in the chemical composition associated with decomposition processes, we analysed the C-storage of leaves harvested in February 2010 for 100% defoliated *N. betuloides* and compared it to the C-storage found in dead leaves. To determine C- (soluble sugars and starch) and N-storage concentrations and pools, a small, coarse piece of superficial root (approximately 0.5–1 cm diameter) and main stem (collected at middle height) were taken from each juvenile, and bark and phloem were removed in the field with a knife. Plant material was brought to the laboratory in a cooler box with an ice pack to reduce tissue respiration for the 2 h trip. Samples for C- and N-storage analyses were heated in a microwave in three 20 s cycles at maximum power in order to stop enzymatic activity (Popp *et al.* 1996). Samples were then dried in a forced-air stove at 70 °C for 72 h, and afterwards, they were ground to a fine powder by a coffee mill and a mortar, and finally, they were stored at 4 °C until chemical analysis. For biomass distribution estimation, four juveniles per treatment were excavated. Roots, main stem and all leaves were collected separately. In the laboratory, roots were thoroughly washed with tap water, gently brushed and put back in paper bags, along with leaves and stems. All plant material was placed to dry at 70 °C for 72 h and then dry weights were recorded.

CHEMICAL ANALYSES

Leaves, stem and roots of each juvenile were analysed for non-structural carbohydrates (NSC = soluble sugars + starch) and N concentrations. In approximately 15 mg of dried powder sample, soluble sugars and starch concentrations were determined. Soluble sugars were extracted with a methanol:chloroform:water solution, separated from pigments and lipids by adding water and chloroform (Rose *et al.* 1991), and main sugars (sucrose, glucose, and fructose) determined with the phenol sulphuric method, using 2% phenol and reading at 490 nm (Chow & Landhäusser 2004). The residual pellet was dried overnight at 50 °C in a forced-air stove and starch was then gelatinized (Rose *et al.* 1991) and hydrolysed to glucose with amyloglucosidase (Sigma-Aldrich 10115, St. Louis, MO, USA) at 45 °C overnight. Glucose was determined similarly to soluble sugars (Chow & Landhäusser 2004). Soluble sugars and starch concentrations were expressed as mg per gram of dry weight. Total NSC concentrations were estimated from the sum of soluble sugars and starch. The nitrogen concentration was determined in 25 mg of dry and ground samples of leaves, stem and roots, by a combustion analyser (LECO TruSpec® Micro CHN; Center of Applied Isotope Studies of the Uni-

versity of Georgia, Athens, GA USA). N concentrations were expressed both per mass unit (as % of dry matter) and per area unit (in the case of leaves). Carbohydrate and N pools were obtained by multiplying the biomass of each tissue by NSC and N concentrations.

STATISTICAL ANALYSES

The influence of defoliation intensity and species foliar habit in biomass distribution, soluble sugars, starch, NSC and N concentrations and pools were analysed fitting linear mixed-effects models (LMM). In the modelling, we considered the defoliation level (0%, 50% and 100%) and species foliar habit (deciduous and evergreen) as fixed factors and sampling points as random factors to account for among-point variation. To test whether there was a difference between deciduous and evergreen species' C- and N-storage across defoliation levels, we computed mean values, confidence intervals, and predicted differences between deciduous and evergreen species, and inference (*t*- and *P*-value) on planned contrasts using the *esticon* function in the doBy package (Højsgaard *et al.* 2012). For 100% defoliated *N. betuloides*, leaf carbohydrate concentrations in early February 2010 (living leaves) and mid-December 2010 (dead leaves) were compared with a Student's *t*-test. We planned to evaluate survival responses in the different treatments by fitting generalized LMM, considering a logistic link function (alive vs. dead, binomial residual distribution; Pinheiro & Bates 2001). However, we found no variation in survival (complete mortality for one treatment, and complete survival for the other five treatments; see Results below). Thus, we only show absolute values demonstrating the blatant difference among treatments. Finally, cumulative regrowth was compared between species at the end of the experiment for the two defoliation treatments (50% and 100% defoliation) using LMMs, where species was the fix factor and sampling point was the random factor. All analyses were done in R version 2.15.3 (R Development Core Team 2013).

Results

By the time the second complete defoliation was carried out (mid-summer 2010, i.e. February), all 60 juvenile trees were found alive. At this time, regrowth was very low in *N. pumilio* (19% of initial leaf biomass) and higher in *N. betuloides* (42%) (Fig. 2). In the second growing season (specifically by mid-December 2010), all *N. betuloides* juveniles (evergreen) that had been subjected to 100% defoliation (two consecutive 100% defoliations during the first growing season) were found dead, with a few attached leaves formed in the prior growing season (Fig. 2). In contrast, no mortality was observed in *N. pumilio* (deciduous) under the same defoliation regime during the experiment. Furthermore, this species was able to regrow throughout the 3 years of the experiment (Fig. 2). Since the first pulse of regrowth in *N. betuloides* was high, cumulative regrowth was statistically similar between species that underwent 100% defoliation. We found no juvenile mortality of any species with 50% defoliation at any time in the 3 year experiment. Cumulative regrowth for this defoliation intensity was also similar between species (Fig. 2).

In the absence of defoliation (i.e. controls), leaf NSC concentrations were similar between species, although starch was higher in *N. betuloides*, whereas soluble sugars were higher
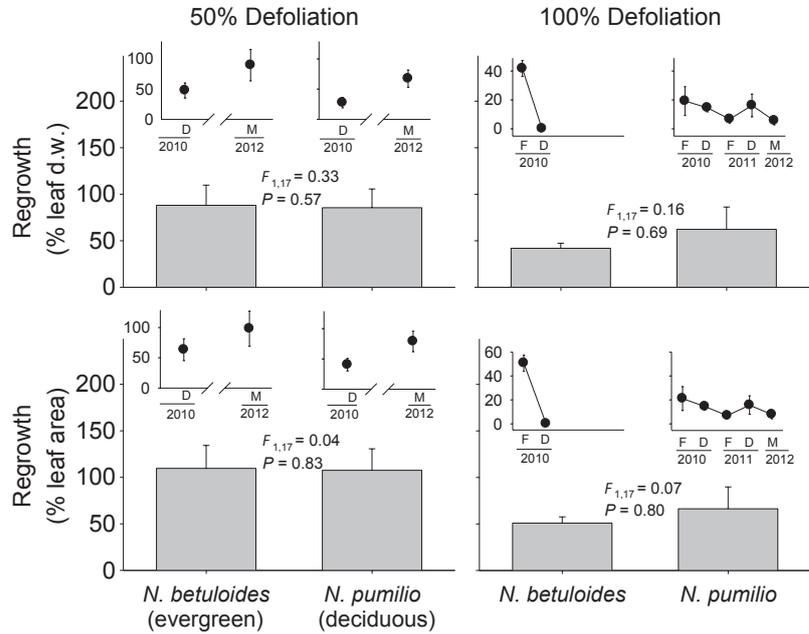
Fig. 2. Cumulative (bars) and seasonal (dots, inset graphs) regrowth in juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous) subjected to 50% and 100% defoliation intensity during three growing seasons in a mixed forest in Patagonia, Chile. For cumulative regrowth, values represent the sum of successive regrowth relative to the initial total leaf mass and leaf area of each juvenile tree. *F*-ratios, degrees of freedom and *P*-values based on linear mixed-effects models (LMM) analyses are for the foliar habit comparison. D: December; F: February; M: March.

Table 1. Statistical results (*F*- and *P*-value) based on linear mixed-effects models (LMM) showing the effects of species (spp.), defoliation level (*D*) and the interaction of both on concentrations of non-structural carbohydrates (NSC), starch, soluble sugars and nitrogen (N) in leaves, stems and roots (in mg g^{-1} d.w.) for juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous) in Patagonia, Chile

	Soluble sugars	Starch	NSC	N
Concentration				
Leaves				
sp.	64.92 (<0.001)	93.04 (<0.001)	22.38 (<0.001)	12.99 (0.002)
D	11.52 (<0.001)	149.74 (<0.001)	8.26 (0.001)	10.20 (0.012)
sp. × *D*	7.36 (0.002)	122.67 (<0.001)	2.83 (0.072)	1.97 (0.171)
Stems				
sp.	13.09 (<0.001)	2.81 (0.101)	1.47 (0.232)	4.18 (0.051)
D	20.95 (<0.001)	31.90 (<0.001)	42.39 (<0.001)	7.02 (0.0036)
sp. × *D*	14.79 (<0.001)	1.51 (0.233)	4.07 (0.024)	6.20 (0.007)
Roots				
sp.	8.59 (0.005)	33.08 (<0.001)	35.00 (<0.001)	0.83 (0.372)
D	9.54 (<0.001)	45.64 (<0.001)	41.57 (<0.001)	3.47 (0.047)
sp. × *D*	5.11 (0.010)	3.80 (0.030)	5.55 (0.007)	4.50 (0.022)
Pool				
Leaves				
sp.	3.71 (0.067)	7.37 (0.011)	3.13 (0.085)	21.24 (<0.001)
D	62.78 (<0.001)	112.11 (<0.001)	120.79 (<0.001)	23.77 (<0.001)
sp. × *D*	9.69 (<0.001)	6.42 (0.005)	5.15 (0.011)	17.27 (<0.001)
Stems				
sp.	6.06 (0.018)	1.56 (0.220)	0.45 (0.506)	1.39 (0.25)
D	23.36 (<0.001)	28.37 (<0.001)	29.10 (<0.001)	5.79 (0.008)
sp. × *D*	4.78 (0.014)	3.50 (0.040)	2.56 (0.091)	5.37 (0.010)
Roots				
sp.	0.002 (0.888)	22.52 (<0.001)	15.64 (<0.001)	3.16 (0.087)
D	2.51 (0.094)	23.65 (<0.001)	17.99 (<0.001)	0.07 (0.929)
sp. × *D*	2.77 (0.075)	1.97 (0.153)	2.34 (0.108)	0.08 (0.011)

in *N. pumilio* (Table 1, Fig. 3). NSC concentrations in stems were also similar between species; however, in roots, *N. pumilio* had significantly higher concentrations of starch and NSC than *N. betuloides* (Table 1, Fig. 3). The effect of defoliation on NSC concentrations differed between species and depended on tissue type. First, according to our expecta-tion that under complete defoliation *N. pumilio* incurs greater C and N reductions in woody tissues than *N. betuloides*, we effectively found that for roots, control trees of *N. pumilio* had higher NSC (starch and sugar) concentrations than *N. betuloides* counterparts, while both species had similar NSC concentrations under 100% defoliation. This pattern
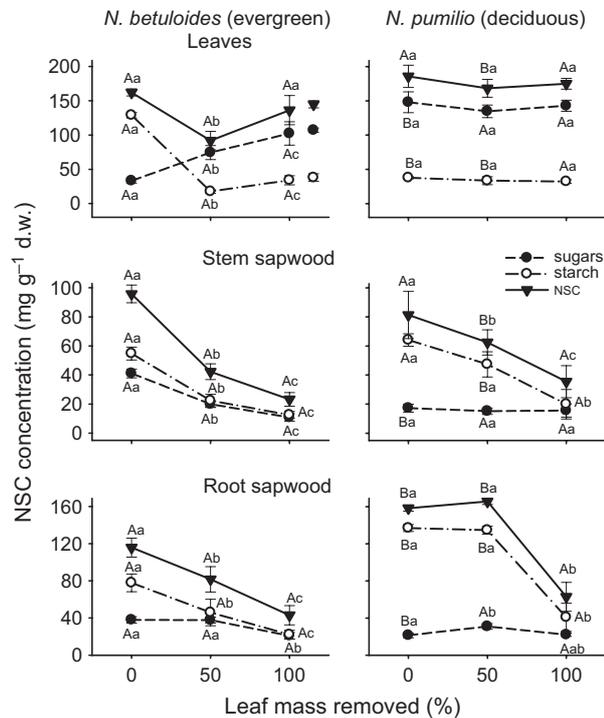
Fig. 3. Soluble sugars (closed circles), starch (open circles) and total non-structural carbohydrate (NSC = soluble sugars + starch) (closed triangles) concentrations in leaves, stems and roots of juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous) subjected to different defoliation levels (0%, 50% and 100%) in a mixed temperate forest in Patagonia, Chile. Chemical concentrations were determined after three growing seasons, except in 100% defoliated *N. betuloides* individuals, where they were determined after one growing season (December 2010). Detached right-sided symbols in the top-left figure show leaf concentrations of soluble sugars, starch and NSC of 100% defoliated *N. betuloides* in early February 2010. Different upper case letters indicate significant ($P < 0.05$) differences in concentrations between species, and different lower case letters indicate significant differences among treatments within species.

shows that *N. pumilio* experienced a higher reduction in root C-storage than *N. betuloides* (Table 1, Fig. 3). Following the same rationale, however, there was no interspecific difference

in the stems' C-storage mobilization in response to 100% defoliation. This result, along with the similar root NSC concentrations between species under 100% defoliation, led to partial support for our second expectation, that is, that *N. betuloides* may run out of reserves under complete defoliation and die. Third, we had also expected that under partial defoliation (50%), no interspecific difference in C mobilization should be observed. We found, however, that *N. pumilio* maintained higher concentrations of starch and NSC than *N. betuloides* in woody tissues (stems and roots) and leaves when subjected to 50% defoliation (Table 1, Fig. 3). Finally, for 100% defoliated *N. betuloides* individuals, leaves collected in February 2010 had similar soluble sugars ($F_{1,12} = 0.29$; $P = 0.60$), starch ($F_{1,12} = 0.12$; $P = 0.73$) and NSC ($F_{1,12} = 0.52$; $P = 0.52$) concentrations as dead leaves collected in mid-December 2010.

For controls, *N. betuloides* had significantly more biomass invested in leaves than *N. pumilio* ($F = 71.46$, $P < 0.0001$, Table 2), but stem and root biomass did not differ between species ($F = 1.32$, $P = 0.26$; $F = 1.66$, $P = 0.17$, respectively, Table 2). Defoliation had obvious major effects on leaf biomass ($F = 53.3$, $P < 0.0001$), but not on wood biomass ($F = 1.71$, $P = 0.19$; $F = 0.37$, $P = 0.69$, in stems and roots, respectively). The reduction of leaf biomass caused by the defoliation was more pronounced in *N. betuloides* than in *N. pumilio* ($F = 28.75$, $P < 0.0001$) (Table 2). Total plant biomass was similar between species ($F = 2.66$; $P = 0.113$) and was equally reduced with the defoliation intensity in both species (species: $F = 4.13$; $P = 0.027$, species*defoliation: $F = 1.38$; $P = 0.27$). Total amounts of NSC, starch and soluble sugars per tissue (i.e. pools, the carbohydrate concentrations of a given tissue by the biomass of that tissue) followed similar trends to those found for their respective concentrations. In controls, juvenile leaves' NSC pools were higher in *N. betuloides* than in *N. pumilio* (Fig. 4).

Under control conditions, *N. pumilio* had significantly higher N concentrations per mass unit than *N. betuloides* across the different tissues (Table 1 and Fig. 5). For leaves, *N. pumilio* also had higher N concentrations per area unit than *N. betuloides* ($F = 10.41$; $P = 0.004$). A similar pattern

Table 2. Mean values (±1 SE) for leaves, stems, roots and total biomass of juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous) subjected to different defoliation intensities (0%, 50% and 100%) under field conditions in Patagonia, Chile. Biomasses were determined after three growing seasons, except in 100% defoliated *N. betuloides* where they were determined after one growing season (December 2010)

Defoliation (%)	Leaves	Stems	Roots	Total
	Nothofagus betuloides			
0	42.0 (±1.2) a	115.0 (±12.2) a	55.6 (±2.2) a	213.1 (±5.6) a
50	66.0 (±12.7) b	78.9 (±25.0) a	42.1 (±10.6) a	187.1 (±34.2) ab
100	0.22 (±0.12) c	117.0 (±8.2) a	58.6 (±3.4) a	175.6 (±9.50) b
	Nothofagus pumilio			
0	14.5 (±2.0) a	110.7 (±13.7) a	57.0 (±8.5) a	194.62 (±21.7) a
50	8.9 (±2.2) b	98.0 (±21.6) a	81.0 (±18.5) a	186.30 (±31.5) ab
100	1.06 (±0.4) c	55.8 (±7.8) a	46.0 (±9.8) a	102.89 (±17.4) b

Values are given in grams of dry weight. Different lower case letters indicate significant differences among treatments at $P < 0.05$.
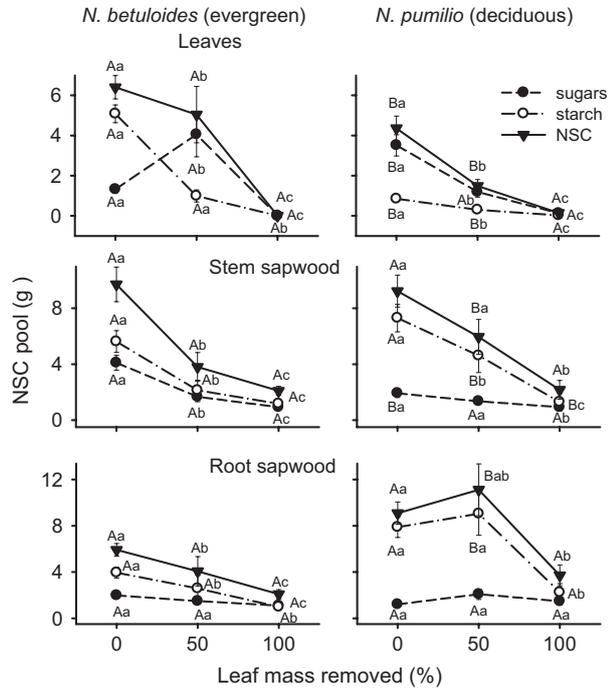
Fig. 4. Soluble sugars (closed circles), starch (open circles) and total non-structural carbohydrate (NSC = soluble sugars + starch) (closed triangles) pools in leaves, stems and roots of juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous) subjected to three different defoliation levels (0%, 50% and 100%) in a mixed temperate forest in Patagonia, Chile. Chemical concentrations were determined after three growing seasons, except in 100% defoliated *N. betuloides* where they were determined after one growing season (December 2010). Different upper case letters indicate significant (*P* < 0.05) differences in concentrations between species, and different lower case letters indicate significant differences among treatments within species.

Fig. 5. Nitrogen (N) concentrations (left panel) and pools (right panel) in leaves, stems and roots of juvenile trees of *Nothofagus betuloides* (evergreen) and *Nothofagus pumilio* (deciduous) subjected to different defoliation levels (three levels: 0%, 50% and 100%) in a mixed temperate forest in Patagonia, Chile. Chemical concentrations were determined after three growing seasons, except in 100% defoliated *N. betuloides* where they were determined after one growing season (December 2010). Pools refer to the total amount of N of a given tissue, and they were calculated as the product between the N concentration and the total biomass of that tissue. Different upper case letters indicate significant (*P* < 0.05) differences in concentrations between species, and different lower case letters indicate significant differences among treatments within species.

was observed for N pools, with the exception of leaves where N pools were higher in *N. betuloides* due to its higher leaf biomass. Defoliation had no effect on the N concentrations of *N. betuloides* leaves and stems, but it caused a significant increase (proportional to defoliation intensity) in the roots' N concentrations (Fig. 5). In *N. pumilio*, defoliations provoked a significant increase in leaves' N concentrations per mass unit, but not per leaf area unit (Table 1), and a significant decrease in stems and roots' N concentrations (Table 1 and Fig. 5). With the exception of leaves, trends in N pools were generally similar to those in N concentrations.

Discussion

DECIDUOUSNESS AND TOLERANCE TO DEFOLIATION

The deciduous species *N. pumilio* had higher NSC and N concentrations (and pools) for stem and root tissues than the evergreen *N. betuloides*. The difference in terms of roots' NSC reached approximately 40% and 50% for concentrations and pools, respectively; higher differences were found for N concentrations and pools. *Nothofagus betuloides*, in contrast, had higher NSC and N pools in leaves due to its higher leaf
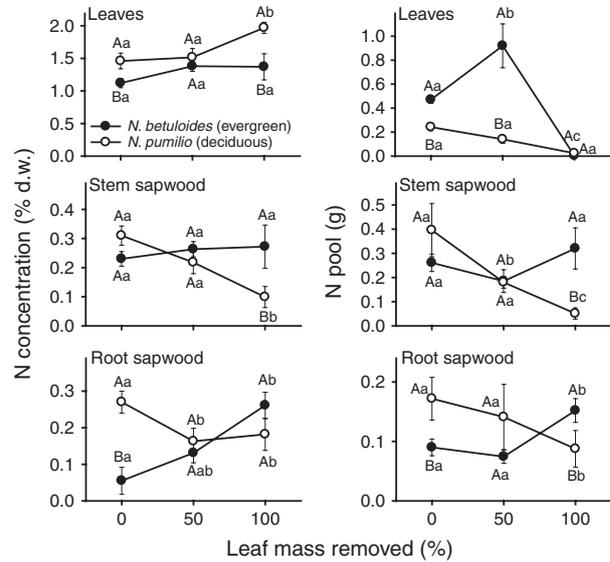
biomass. These results corroborate the pattern reported in classical and more recent studies (Kozlowski 1992; Vanderklein & Reich 1999; Hoch, Richter & Körner 2003; Fajardo, Piper & Hoch 2013; Richardson *et al.* 2013). Whereas most previous studies only worked with one tissue (usually branches or shoots), ours is the first to consider the whole-plant's C and N-storage.

One of our study's key findings was that the deciduous tree species, *N. pumilio*, was more tolerant to complete and recurrent defoliations than the evergreen species *N. betuloides*, which, in contrast, died after two complete defoliations in only one growing season. In total, *N. pumilio* was completely defoliated five times during three growing seasons and kept regrowing ultimately surviving until the end of the experiment. To the best of our knowledge, this capability to regrow and survive complete and successive defoliations has not been previously documented for a tree species. As hypothesized, the regrowth and survival of *N. pumilio* in response to complete defoliation were associated with a significant decrease of C- and N-storage (concentrations and pools in stems and roots). Complete defoliation also reduced the amount of C stored in *N. betuloides*' stems and roots (but not N), although the decrease in absolute values of the roots' NSC concentrations was smaller than in *N. pumilio* due to lower initial NSC concentrations (Fig. 3). This suggests that *N. pumilio* incurs a stronger mobilization of C- and N-storage

than *N. betuloides* to promptly replace foliage tissue and eventually survive. The mobilization of C and N stored in woody tissues very likely allowed *N. pumilio* to achieve compensatory photosynthesis, as suggested from the leaves' increased N concentrations (Fig. 5) and the well-known positive relationship between leaf N and photosynthetic capacity (Reich *et al.* 1999). It is important to note, however, that the relative decrease in roots' starch and NSC concentrations (i.e. the proportional decrease relative to control concentrations) was rather similar between species (62.89% for *N. betuloides* and 60.27% for *N. pumilio*). Thus, absolute changes in C-storage were better predictors of defoliation tolerance than relative ones. The storage of C and N in woody tissues is one of the most conspicuous traits differentiating winter deciduous from evergreen species in temperate forests (Grelet *et al.* 2001; Millard *et al.* 2001; Hoch, Richter & Körner 2003; Fajardo, Piper & Hoch 2013) and so far no satisfactory explanation has accounted for this pattern. It has been proposed that the differential C- and N-storage sites in evergreen and deciduous temperate tree species indicate different tolerances to defoliation (Millard *et al.* 2001; Hoch, Richter & Körner 2003); our results support this proposal if defoliation is complete and recurrent, but not if it is partial.

Our second expectation, that is, that *N. betuloides* would run out of reserves under complete defoliation, and that this, in turn, would lead to death, was partially supported. *Nothofagus betuloides* did not overcome two complete defoliations (in the first growing season) and died, but its final levels of C- and N-storage were not significantly lower than those of *N. pumilio* after 3 years of defoliation (Figs 3 and 5). Given that completely defoliated *N. betuloides* trees had to be analysed for C- and N-storage as *dead* individuals in late spring, while their *N. pumilio* counterparts were analysed as planned in late summer, and 2 years later, differences in harvest dates and tissue conditions could have led to misestimates for the amount of C stored in *N. betuloides*. Arguing against this possibility, we found similar concentrations of soluble sugars, starch and NSC in dead leaves and leaves harvested from the same individuals at the previous defoliation (Fig. 3). Given this result and knowing that the study site experiences snow and low temperatures throughout most of the winter, we assume that tissue decomposition of dead *N. betuloides* before harvest was negligible. The question is then, how could *N. pumilio* survive with such *low* C and N content, while *N. betuloides* could not? We think that *N. pumilio* had a more conservative strategy than *N. betuloides*, given that it never refoliated more than approximately 20% of its original canopy. In contrast, *N. betuloides* refoliated approximately 42% before it died (Fig. 2). Thus, the same level of C-storage was achieved from a rationed regrowth (i.e. evenly distributed in time) with leaves photosynthetically more efficient (i.e. higher leaf N) in *N. pumilio* and from a larger regrowth with leaves photosynthetically less efficient (i.e. lower leaf N) in *N. betuloides*. *Nothofagus betuloides*' strategy could make C and nutrients less available to tolerate subsequent stressful events (e.g. defoliations, freezing). It may not be a coincidence that *N. betuloides* died in the winter, when the demand for

osmotic agents – including sugars and derived compounds – to tolerate freezing increases significantly in *Nothofagus* species of southern South America (Reyes-Díaz *et al.* 2005). This explanation predicts that *N. pumilio* had more C-storage than *N. betuloides* after the first growing season and that it might have been close to dying after three growing seasons when its C-storage level was similar to the one reached by the dead *N. betuloides* individuals. Another potential explanation for *N. betuloides*' complete mortality is that, as an evergreen species, it is adapted to maintain active transpiration throughout the winter, while *N. pumilio*, as a deciduous species, experiences annual cycles of embolism and reactivation of its xylem (Sperry *et al.* 1994). Thus, *N. betuloides* may have died in the winter due to an irreversible damage (i.e. loss of functionality) to its vascular system (xylem and phloem) caused by the cessation of the water flux in low temperature conditions. Given the interdependence of phloem and xylem fluxes, impeded hydraulic conductance is expected to impair the phloem transport (Sala, Piper & Hoch 2010; Sala, Woodruff & Meinzer 2012), which in turn could limit the mobilization and translocation of stored resources and the hormonal production and signalization (Kulman 1971; Richards 1993). In fact, the apparent lack of N mobilization from roots or stems in completely defoliated *N. betuloides* juveniles is consistent with the previous idea. In contrast, the deciduous habit (i.e. *N. pumilio*) might be associated with a vascular system adapted to leaf loss, not only driven by phenology, but also by disturbances (e.g. massive defoliations).

We also expected that partial defoliations should not induce differential responses in the C- and N-storage or the survival of deciduous and evergreen species. Our study's results supported this expectation; both species were similarly able to survive well (no mortality) and to regrow. These treatments, however, caused greater reductions in stem and root C-storages in *N. betuloides* than in *N. pumilio*. In fact, *N. pumilio* roots did not reduce their NSC concentrations or pools and this species experienced only a small NSC decrease (concentration and pool) in its stem (Figs 3 and 4). We think that the similar survival responses to partial defoliation for both species reflect different mechanisms. In deciduous species, the fast C-autonomy reached by new leaves and compensatory photosynthesis promote a rapid refilling of storages used to build up new leaves (Palacio *et al.* 2008; Keel & Schädel 2010; Landhäusser 2011). In evergreen species, on the other hand, remaining leaves provide the energy and resources required to regrow (Vanderklein & Reich 1999; Millard *et al.* 2001; Li, Hoch & Körner 2002), which explains why evergreens usually tolerate partial defoliations, but are extremely sensitive to complete defoliation (Craighead 1940; Krause & Raffa 1996; Mundim *et al.* 2012). We conclude that deciduous species are more adapted than evergreens to complete and repeated defoliations, but this *advantage* disappears in situations of partial defoliation.

The hypothesis of higher herbivory tolerance in deciduous than in evergreen species has been proposed previously (Bryant, Chapin & Klein 1983; Hoch, Richter & Körner 2003; Millard & Grelet 2010), but our study adds a key

empirical piece of evidence not yet considered: the intensity and frequency of defoliation. The reformulated hypothesis, that is, that higher C- and N-storage in woody tissues of deciduous species promotes better tolerance to complete and repeated defoliation compared to evergreen species, is consistent with the view of herbivory tolerance as the selected mechanism under frequent, intense and often unpredictable defoliations (van der Meijden, Wijn & Verkaar 1988; Rosenthal & Kotanen 1994; Haukioja & Koricheva 2000). This hypothesis is also consistent with the high intensity of defoliation under which temperate deciduous species, with more tender leaves than evergreens, might have evolved. Complete defoliations driven by herbivores can occur repeatedly, during consecutive years (Rose 1958; Kulman 1971), or even in the same season (Wilkinson, Becker & Benjamin 1966; Freelich 2002), when they can also co-occur with severe abiotic defoliation (Rose 1958; Veblen *et al.* 1996; Freelich 2002; Lorenz & Becher 2012).

Our results could help to understand biogeographic patterns of *Nothofagus* species in southern South America associated with leaf habit. Climate, with a major focus on drought, low temperatures and soil nutrition, has been the main factor linking plant distribution and leaf lifespan (Chabot & Hicks 1982; Givnish 2002; McGlone *et al.* 2004). Yet, a satisfactory explanation for the ample, large-scale dominance of deciduous *Nothofagus* species in southern South America, across different climates (Mediterranean, Temperate and Subantarctic) and soil types (old in the Coastal Range, young volcanic in the Andes Cordillera), is still lacking. The dominance of *N. pumilio* as a deciduous tree line species for most of the southern Andes of South America (35–55°S), across temperature and precipitation gradients (Fajardo, Piper & Cavieres 2011), as opposed to the dominance of evergreen conifers in the northern hemisphere, is still not well understood. One explanation has pointed to the lack of the 'right taxon' (e.g. an evergreen conifer; Körner & Paulsen 2004), but this idea has not been supported by empirical studies (Fajardo, Piper & Cavieres 2011; Fajardo & Piper 2014). Selection for the deciduous leaf habit to face complete and periodic defoliations could be an alternative explanation for the dominance of *N. pumilio* at high elevations across all the southern Andes of South America (Fajardo, Piper & Hoch 2013). Forests of *N. pumilio* are frequently exposed to insect outbreaks – particularly *O. amphimone* (Lepidoptera: Saturniidae) – and it is thought that they have evolved along with these outbreaks (Paritsis 2009; Paritsis & Veblen 2010; Fig. 1). In addition, temperate forests of southern South America, and *Nothofagus* forests in particular, are thought to have evolved under intense pressure of large-scale abiotic disturbances (Veblen & Ashton 1978; Grubb *et al.* 2013), which can be expected to more severely defoliate deciduous than evergreen species. It is thus highly probable that *N. pumilio* is adapted to abiotic-driven defoliations, in general. Consistent with this, it has been reported that intense summer drought causes premature leaf abscission in deciduous *Nothofagus* species which nonetheless are better able than evergreen *Nothofagus* to survive such events (Veblen *et al.* 1996).

Conclusions

Our study suggests that the winter deciduous habit entails advantages when defoliation is intense. These advantages are linked, in part, to higher C- and N-storage in woody tissues, but other mechanisms linked to deciduousness itself seem to be involved as well. The absolute mortality observed in the intensively defoliated evergreen *Nothofagus*, but not in the deciduous *Nothofagus*, could be related to irreversible damage to the vascular system (xylem and phloem) caused by the cessation of the water flux in low temperature conditions (i.e. winter time). We suggest that explanations of global distribution patterns of winter deciduous and evergreen tree species should consider the possibility of massive outbreak episodes and abiotic disturbances across different climates, latitudes or soil types. Where leaf damage due to heavy herbivory, frost, falling debris, avalanches and other disturbances is frequent, the deciduous leaf habit can be expected to be more successful than its evergreen counterpart. We believe our study is important because it dealt with two sympatric, congeneric, different foliar habit species which reduces any potential phylogenetic noise. Nonetheless, further studies are necessary to determine whether the trends found in this study do indeed represent an adaptive functional link.

Acknowledgements

References

Alberdi, M., Romero, M., Rios, D. & Wenzel, H. (1985) Altitudinal gradients of seasonal frost resistance in *Nothofagus* communities of southern Chile. *Acta oecologica. Oecologia plantarum*, **6**, 21–30.

Barbaroux, C. & Bréda, N. (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiology*, **22**, 1201–1210.

Boscagli, A. (1982) The starch content in *Fraxinus ornus* L. during the yearly cycle. Histological observations. *Giornale Botanico Italiano*, **116**, 41–49.

Bryant, J.P., Chapin, F.S. III & Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357–368.

Chabot, B.F. & Hicks, D.J. (1982) The ecology of leaf life spans. *Annual Review of Ecology and Systematics*, **13**, 229–259.

Chow, P.S. & Landhäusser, S.M. (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*, **24**, 1129–1136.

Craighead, F.C. (1940) Some effects of artificial defoliation on pine and larch. *Journal of Forestry*, **38**, 885–888.

Dickson, R.E. (1989) Carbon and nitrogen allocation in tree. *Annals of Forest Science*, **46**, 631–647.

Dobbs, R.C. & McMinn, R.G. (1973) Hail damage to a new white spruce and lodgepole pine plantation in central British Columbia. *The Forestry Chronicle*, **49**, 174–175.

El Zein, R., Maillard, P., Bréda, N., Marchand, J., Montpied, P. & Gérant, D. (2011) Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. *Tree Physiology*, **31**, 843–854.

Fajardo, A. & Piper, F.I. (2014) An experimental approach to explain the southern Andes elevational treeline. *American Journal of Botany*, **101**, 788–795.

Fajardo, A., Piper, F.I. & Cavieres, L.A. (2011) Distinguishing local from global climate influences in the carbon status variation with altitude of a treeline species. *Global Ecology and Biogeography*, **20**, 307–318.

Fajardo, A., Piper, F.I. & Hoch, G. (2013) Similar variation in carbon storage between deciduous and evergreen treeline species across elevational gradients. *Annals of Botany*, **112**, 623–631.

Frelich, L.E. (2002) The disturbance regime and its components. *Forest Dynamics and Disturbance Regimes* (ed. L.E. Freelich), pp. 15–43. Cambridge University Press, Cambridge, UK.

Givnish, T.J. (2002) Adaptive significance of evergreen vs. deciduous leaves: solving the triple paradox. *Silva Fennica*, **36**, 703–743.

Grayburn, A.W. (1957) Hail damage to exotic forests in Canterbury. *New Zealand Journal of Forestry*, **7**, 1950–1957.

Grelet, G.A., Alexander, I.J., Proe, M.F., Frossard, J.S. & Millard, P. (2001) Leaf habit influences nitrogen remobilization in *Vaccinium* species. *Journal of Experimental Botany*, **52**, 993–1002.

Grubb, P.J., Bellingham, P.J., Koyhama, T., Piper, F.I. & Valido, A. (2013) Disturbance regimes, gap-demanding trees and seed mass related to tree height in warm temperate rain forests worldwide. *Biological Reviews*, **88**, 701–744.

Handa, I.T., Körner, C. & Hättenschwiler, S. (2005) A test of the treeline carbon limitation hypothesis by in situ CO_2 enrichment and defoliation. *Ecology*, **86**, 1288–1300.

Haukioja, E. & Koricheva, J. (2000) Tolerance to herbivory in woody vs. herbaceous plants. *Evolutionary Ecology*, **14**, 551–562.

Hevia, F., Minoletti, O.M.L., Decker, K.L. & Boerner, R.E. (1999) Foliar nitrogen and phosphorus dynamics of three Chilean *Nothofagus* (Fagaceae) species in relation to leaf lifespan. *American Journal of Botany*, **86**, 447–455.

Hoch, G., Richter, A. & Körner, C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment*, **26**, 1067–1081.

Højsgaard, S., Halekoh, U., Robinson-Cox, J., Wright, K. & Leidi, A. (2012) *Groupwise Summary Statistics, General Linear Contrasts, Population Means (Least-Square-Means), and Other Utilities*. R Package Version 4.5-3 available at http://cran.open-source-solution.org/web/packages/doBy/doBy.pdf.

Keel, S.G. & Schädel, C. (2010) Expanding leaves of mature deciduous forest trees rapidly become autotrophic. *Tree Physiology*, **30**, 1253–1259.

Körner, C. & Paulsen, J. (2004) A world-wide study of high altitude treeline temperatures. *Journal of Biogeography*, **31**, 713–732.

Kozlowski, T.T. (1992) Carbohydrate sources and sinks in woody plants. *Botanical Review*, **58**, 107–222.

Krause, S. & Raffa, K. (1996) Differential growth and recovery rates following defoliation in related deciduous and evergreen trees. *Trees*, **10**, 308–316.

Kulman, H.M. (1971) Effects of insect defoliation on growth and mortality of trees. *Annual Review of Entomology*, **16**, 289–324.

Landhäusser, S. (2011) Aspen shoots are carbon autonomous during bud break. *Trees*, **25**, 531–536.

Li, M., Hoch, G. & Körner, C. (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees*, **16**, 331–337.

Lorenz, M. & Becher, G. (2012) *Forest Condition in Europe, 2012 Technical Report of ICP Forests*. Work Report of the Thünen Institute for World Forestry 2012/1. *ICP Forests*.

Mataa, M. & Tominaga, S. (1998) Effects of root restriction on tree development in ponkan mandarin (*Citrus reticulata* Blanco). *Journal of the American Society for Horticultural Science*, **123**, 651–655.

Mataa, M., Tominaga, S. & Kozaki, I. (1998) The effect of time of girdling on carbohydrate contents and fruiting in ponkan mandarin (*Citrus reticulata* Blanco). *Scientia Horticulturae*, **73**, 203–211.

McGlone, M.S., Dungan, R.J., Hall, G.M.J. & Allen, R.B. (2004) Winter leaf loss in the New Zealand woody flora. *New Zealand Journal of Botany*, **42**, 1–19.

van der Meijden, E., Wijn, M. & Verkaar, H.J. (1988) Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, **51**, 355–363.

Millard, P. (1994) Measurement of the remobilization of nitrogen for spring leaf growth of trees under field conditions. *Tree Physiology*, **14**, 1049–1054.

Millard, P. & Grelet, G.A. (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiology*, **30**, 1083–1095.

Millard, P., Hester, A., Wendler, R. & Baillie, G. (2001) Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Functional Ecology*, **15**, 535–543.

Miller, S.A., Smith, G.S., Boldingh, H.l. & Johansson, A. (1998) Effects of water stress on fruit quality attributes of kiwifruit. *Annals of Botany*, **81**, 73–81.

Mooney, H.A. & Gulmon, S.L. (1982) Constraints on leaf structure and function in reference to herbivory. *BioScience*, **32**, 198–206.

Mundim, F.M., Bruna, E.M., Vieira-Neto, E.H.M. & Vasconcelos, H.L. (2012) Attack frequency and the tolerance to herbivory of neotropical savanna trees. *Oecologia*, **168**, 405–414.

Myers, J.A. & Kitajima, K. (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology*, **95**, 383–395.

Ozolincius, R. & Stakenas, V. (1996) Tree crown defoliation: influencing factors. *Baltic Forestry*, **2**, 48–55.

Palacio, S., Hester, A.J., Maestro, M. & Millard, P. (2008) Browsed *Betula pubescens* trees are not carbon-limited. *Functional Ecology*, **22**, 808–815.

Pallardy, S.G. (2007) *Physiology of Woody Plants*. Academic Press, San Diego, CA.

Paritsis, J. (2009) *Insect Defoliator Outbreaks and Environmental Heterogeneity in Nothofagus Forests in the Patagonian Andes*. University of Colorado, Boulder, CO.

Paritsis, J. & Veblen, T.T. (2010) Dendroecological analysis of defoliator outbreaks on *Nothofagus pumilio* and their relation to climate variability in the Patagonian Andes. *Global Change Biology*, **17**, 239–253.

Paritsis, J., Elgueta, M., Quintero, C. & Veblen, T.T. (2010) New host-plant records for the defoliator *Ormiscodes amphimone* (Fabricius) (Lepidoptera: Saturniidae). *Neotropical Entomology*, **39**, 1048–1050.

Pinheiro, J. & Bates, D.M. (2001) *Mixed Effects Models in S and S-PLUS*. Springer, New York.

Popp, M., Lied, W., Meyer, A.J., Richter, A., Schiller, P. & Schwitte, H. (1996) Sample preservation for determination of organic compounds: microwave versus freeze-drying. *Journal of Experimental Botany*, **47**, 1469–1473.

Pringle, E.G., Adams, R.I., Broadbent, E., Busby, P.E., Donatti, C.I., Kurten, E.L., Renton, K. & Dirzo, R. (2011) Distinct leaf-trait syndromes of evergreen and deciduous trees in a seasonally dry tropical forest. *Biotropica*, **43**, 299–308.

R Development Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available at http://www.R-project.org. ISBN 3-900051-07-0.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C. & Bowman, W.D. (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology*, **80**, 1955–1969.

Reyes-Díaz, M., Alberdi, M., Piper, F.I., Bravo, L.A. & Corcuera, L.J. (2005) Low temperature responses of *Nothofagus dombeyi* and *Nothofagus nitida*, two evergreen species from south central Chile. *Tree Physiology*, **25**, 1389–1398.

Richards, J.H. (1993) Physiology of plants recovering from defoliation. *Proceedings of the XVII International Grassland Congress*, pp. 85–94.

Richardson, A.D., Carbone, M.S., Keenan, T.F., Czimczik, C.I., Hollinger, D.Y., Murakami, P., Schaberg, P.G. & Xu, X. (2013) Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytologist*, **197**, 850–861.

Rodríguez, R. & Quezada, M. (2003) Fagaceae. *Berberidaceae-Betulaceae, Flora de Chile*, Vol. 2 (2) (eds C. Marticorena & R. Rodríguez), pp. 64–76. Universidad de Concepción, Concepción.

Rose, A.H. (1958) The effect of defoliation on foliage production and radial growth of quaking aspen. *Forest Science*, **4**, 335–342.

Rose, R., Rose, C.L., Omi, S.K., Forry, K.R., Durall, D.M. & Bigg, W.L. (1991) Starch determination by perchloric acid vs enzymes: evaluating the accuracy and precision of six colorimetric methods. *Journal of Agricultural and Food Chemistry*, **39**, 2–11.

Rosenthal, J.P. & Kotanen, P.M. (1994) Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution*, **9**, 145–148.

Sala, A., Piper, F.I. & Hoch, G. (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist*, **186**, 274–281.

Sala, A., Woodruff, D.R. & Meinzer, F.C. (2012) Carbon dynamics in trees: feast or famine? *Tree Physiology*, **32**, 764–775.

Schädel, C., Blöchl, A., Richter, A. & Hoch, G. (2009) Short-term dynamics of nonstructural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. *Tree Physiology*, **29**, 901–911.

Silla, F. & Escudero, A. (2003) Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species. *Oecologia*, **136**, 28–36.

Sperry, J.S., Nichols, K.L., Sullivan, J.E.M. & Eastlack, S.E. (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology*, **75**, 1736–1752.

Tiffin, P. (2000) Mechanisms of tolerance to herbivore damage: what do we know? *Evolutionary Ecology*, **14**, 523–536.

Tromp, J. (1983) Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. *Plant and Soil*, **71**, 401–413.

Vanderklein, D.W. & Reich, P.B. (1999) The effect of defoliation intensity and history on photosynthesis, growth and carbon reserves of two conifers with contrasting leaf lifespans and growth habits. *New Phytologist*, **144**, 121–132.

Veblen, T. & Ashton, D. (1978) Catastrophic influences on the vegetation of the Valdivian Andes, Chile. *Vegetatio*, **36**, 149–167.

Veblen, T.T., Donoso, C., Kitzberger, T. & Rebertus, A.J. (1996) Ecology of Southern Chilean and Argentinian *Nothofagus* forests. *The Ecology and Biogeography of Nothofagus* Forests (eds T.T. Veblen, R.S. Hill & J. Read), pp. 293–353. Yale University Press, New Haven, CT.

Walters, M.B. & Reich, P.B. (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytologist*, **143**, 143–154.

Wilkinson, R.C., Becker, G.C. & Benjamin, D.M. (1966) The biology of *Neodiprion rugifrons* (Hymenoptera: Diprionidae), a sawfly infesting jack pine in Wisconsin. *Annals of the Entomological Society of America*, **59**, 786–792.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F. *et al.* (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Schematic representation of partial defoliation and detailed explanation of the formula used to calculated regrowth (*R*) in this treatment.