1 # The role of stored carbohydrates and nitrogen in the growth and stress tolerance of planted forest trees

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5 Pedro Villar-Salvador[1*], Mercedes Uscola[2], Douglass F. Jacobs[2]

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7 1. Forest Ecology and Restoration Group, Departamento de Ciencias de la Vida, Universidad
8 de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain
9 2. Department of Forestry and Natural Resources, Hardwood Tree Improvement and
10 Regeneration Center, Purdue University, West Lafayette, IN 47907-2061, USA

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13 *Author for correspondence: email: pedro.villar@uah.es; phone: +34 (91) 8854913

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Abstract

Plants store compounds that supplement external resources to maintain primary functions. We reviewed the role of stored non-structural carbohydrates (NSC) and nitrogen (N) in juvenile woody species for spring growth and cold and drought stress tolerance, which are crucial for early performance of forest plantations. Plant functional types differed in NSC and N partitioning and allocation to new growth. In general, however, new leaves/shoots were more enriched in remobilized resources than new fine roots. Conifers used less remobilized resources than broadleaf species for fine root growth. New shoots/leaves were mostly comprised of remobilized N (>60%) in conifers and broadleaf deciduous species, while broadleaf evergreens relied more on soil N (<50% remobilized N). In contrast, few differences among functional groups existed in the contribution of remobilized carbon (C) (28-45%) to new leaves/shoots, reflecting the importance of current photosynthesis and distinctions in C and N remobilization physiology. Organ source strength for remobilized N was positively related to its contribution to seedling N content. However, leaves are priority N sources in evergreens, which remobilized more N than predicted by their contribution to seedling N content. In contrast, roots in broadleaf evergreens and conifers were poor contributors of remobilized N. Under low stress, spring growth has little effect on NSC reserves. However, prolonged and intense photosynthesis depression strongly reduces NSC. In contrast, N reserves usually decline after planting and their replenishment takes longer than for NSC reserves. Strong storage reduction can hinder seedling stress acclimation and survival capacity. Accumulation of stored resources can be promoted in the nursery by arresting plant growth and supplying resources at a higher rate than seedling growth and maintenance rate. We conclude that the way in which woody plants manage stored resources drives their growth and stress tolerance. However, plant functional types differ in storage physiology, which should be considered in silvicultural management.

Key Words: Cultivation practices, growth, plantation establishment, remobilization, reserves

1) Introduction

Plants consume significant amount of nutrients and energy to maintain their main functions: growth, respiration, reproduction and defense. A large part of these resources is obtained from the soil and atmosphere. However, very often these sources do not meet plant demand because resources have been reduced by competition (Malik and Timmer 1996) or abiotic factors limits their availability in the environment such as extreme soil temperatures or drought (Millard and Grelet 2010). Moreover, main plant functions frequently occur simultaneously (for instance growth and reproduction or concomitantly growth of plant organs), imposing a strong resource demand that plants usually cannot meet by current uptake of external nutrients (Uscola et al. 2015). In these cases, plants rely on stored compounds, which are mobilized to supplement external resources and fulfill plant demand. Storage can be defined as the process by which plants build up compounds that can be mobilized in the future from one organ to another to support main plant functions (Chapin III et al. 1990).

Plant storage can be classified into three categories (Chapin III et al. 1990; Millard and Grelet 2010):

- **Accumulation**, which is the increase in compounds that do not directly promote growth as a result of acquisition exceeding plant demand. This likely is the main storage component in seedlings when subjected to any cultivation practice that slows or stops growth but does not inhibit photosynthesis and nutrient uptake.

- **Reserve formation**, which involves the metabolically regulated synthesis of storage compounds that might otherwise directly promote growth. Compounds can be stored throughout the plant or in particular plant compartments and mobilized to support future growth and functioning of other organs. Reserve formation potentially can compete for resources with other plant functions (Chapin III et al 1990; Palacio et al 2014).

When accumulated compounds or reserves are mobilized to support plant function and growth this physiological process is termed remobilization. The movement of remobilized compounds among tissues is termed translocation (Millard and Grelet 2010).

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93   - **Recycling or resorption**, when compounds that are currently involved in the functioning

94   of a specific organ are withdrawn following breakdown of biochemical machinery and

95   structural components in senescing organs. Resorption mainly affects mineral nutrients

96   and only a small fraction of C is recycled from vegetative organs (Aerts and Chapin III

97   2000). On average, perennial plants recycle around 50% of N and P from senescent

98   leaves, with evergreen woody plants having lower resorption efficiency than deciduous

99   species, although some differences exist among growth forms and plant functional types

100   (Aerts 1996). Recycled nutrients are usually stored and remobilized later to sustain plant

101   functions.

102   Stored "mobile" compounds are of varied nature. Quantitatively and according to their

103   functional relevance, non structural carbon (mainly carbohydrates) (NSC) and nitrogen (N)

104   are the most important stored compounds (Kozlowski 1992; Millard et al. 2007; Millard and

105   Grelet 2010), but plants also store and mobilize significant amounts of mineral nutrients such

106   as potassium, magnesium and phosphorus (Nambiar and Fife 1991; Proe et al. 2000). Most

107   macronutrients can be remobilized but some mineral nutrients such as calcium, iron and

108   heavy metals are not remobilized or recycled (Nambiar and Fife 1987; Chapin III et al. 1990).

109   Levels of stored compounds fluctuate under short-term (hours-days), as in many

110   carbohydrates that vary on a daily time scale (Lambers et al. 2008), seasonal (weeks-month)

111   (Hoch et al 2003a; Körner 2003) and annual time frames (Vargas et al. 2009; Dietze et al.

112   2014). Part of the NSC and N plant pools can also remain immobilized in plant biomass and

113   unavailable for metabolism (Millard et al. 2007; Vizoso et al. 2008). Consequently,

114   "physiological or ecological depletion" of NSC and N does not necessarily mean that organs

115   are completely devoid of these compounds.

116   The amount of stored resources and the way that plants manage them may have

117   adaptive value under specific environmental scenarios. Differences in the amount of stored

118   resources across species has been linked to recovery capacity after catastrophic loss of

119   leaves or roots by disturbances (Cruz et al 2003; Hoffmann et al 2003; Palacio et al 2007)

120   and resisting prolonged stress conditions such as deep shade, low winter temperature and

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121 water stress (Canham et al 1999; Poorter and Kitajima 2007; O'Brien et al 2014). Differences

122 in stored resources can also influence plant performance at an intra-species scale. For

123 instance, the ability of *Sabal palmetto* seedlings to recover from aboveground tissue loss

124 after grazing, burial or fire is related to differences in NSC content among individuals

125 (McPherson and Williams 1998a). Similarly, field survival and growth of planted seedlings in

126 forest restoration projects has been positively related to tissue N and NSC levels both on a

127 concentration and content basis (Marshall 1985; Puttonen 1986; van den Driessche 1991a;

128 Ritchie and Landis 2010). Several physiological processes linked to NSC and N seedling

129 status such as remobilization of stored compounds for new growth, metabolism maintenance

130 and stress tolerance can drive seedling outplanting performance (Puértolas et al. 2010;

131 McDowell 2011; Villar-Salvador et al. 2012).

132   Several reviews have thoroughly addressed the ecological and physiological relevance

133 of NSC (Kozlowski 1992 ; Körner 2003; Millard et al 2007; Dietze et al 2014) and N (Millard

134 1996; Millard and Grelet 2010) storage in plants. Specifically to forestry applications, several

135 studies have also analyzed the utility of NSC and N status as a seedling quality attribute and

136 its utility for predicting seedling outplanting performance in forest plantation projects (Ritchie

137 1984; Marshall 1985; Mattsson 1997). van den Driessche (1991a) addressed how seedling N

138 nutrition in conifers relates to outplanting performance and stress tolerance.

139   Here, we analyze the importance of stored NSC and N for seedling outplanting

140 performance, focusing on two physiological processes linked to storage use by woody plant

141 seedlings: new organ growth and tolerance to drought and frost stress. Specifically we carry

142 out an extensive quantitative and qualitative review on the contribution of remobilized NSC

143 and N for the construction of new organs in spring, which is crucial for seedling

144 establishment and first year survival. We also review the differences in N and NSC

145 distribution among seedlings of distinct functional types in woody plants. Finally, we review

146 how nursery and post-nursery practices affect the NSC and N reserves of planted seedlings

147 and identify gaps of knowledge on storage physiology that deserve future research.

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149   2) **Nature and distribution of NSC and N in seedlings of woody plants**

150   Starch, sucrose, glucose and fructose make up > 80% of NSC in wet temperate trees (Hoch

151   et al. 2003). Raffinose and stachyose are other common NSC in plants although at a much

152   lower concentration than the former sugars. A variety of sugar alcohols, such as sorbitol and

153   pinotol, can also be found in specific taxonomic groups but they are quantitatively less

154   important than common NSC (Kozlowski 1992; Hoch et al. 2003).

155   N is stored as amino acids and specific vegetative proteins, which are formed in the

156   bark after leaf senescence in the fall or during periods of high N uptake for seasonal demand

157   such as in spring growth (Wetzel and Greenwood 1989; Binnie et al 1994; Stepien et al

158   1994; Lambers et al 2008). N can also be stored as NO_3 in fast-growing herb species

159   supplied with high N-NO_3 (Millard 1988). The inactive form of Rubisco can be considered a

160   form of vegetative storage protein and its concentration can strongly increase upon N supply,

161   explaining why N fertilization often does not increase photosynthesis rate in many plants

162   (Valladares et al. 2000; Warren and Adams 2004; Millard et al. 2007). In temperate and cold

163   climate woody plants, other "strictly speaking" vegetative storage proteins are formed in

164   leaves and in the parenchyma cells of bark and wood in stems and roots; their levels strongly

165   increase during the cold season and decrease during bud break (Wetzel and Greenwood

166   1989; Binnie et al. 1994; Stepien et al. 1994).

167   Seedlings and saplings of main functional types in woody plants have important

168   differences in the partitioning of stored NSC and N during winter, with *Quercus* species

169   (oaks) showing distinct partitioning patterns among broadleaf species (Table 1). Overall,

170   broadleaf deciduous seedlings contain more N and NSC in roots than seedlings of broadleaf

171   evergreens and conifers. Among broadleaf deciduous species, *Quercus* species partitioned

172   more stored resources to roots and less to stems than the non-*Quercus* species. Among

173   evergreens, conifers were the functional type that partitioned more N to leaves, and they

174   partitioned more N and NSC in leaves than in the rest of the organs. Broadleaf evergreen

175   non-*Quercus* species partitioned N among main organs in a similar proportion, while

176   broadleaf evergreen *Quercus* species partitioned around three more times N in roots and

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177 leaves than into stems. In relation to NSC, broadleaf evergreen non-*Quercus* species tended
178 to contain more NSC in leaves than in stems and roots, which showed small differences in N
179 partitioning. In contrast, around 70% of NSC was partitioned to roots in broadleaf evergreen
180 *Quercus* species.

181 Variation among functional groups is probably linked to differences in mass partitioning
182 such as reported for winter C partitioning in seedlings of Mediterranean evergreen species
183 (Uscola et al. 2015). For instance, *Quercus* species have large tap roots and consequently,
184 relatively more reserves are allocated to coarse roots. However, some organs such as
185 leaves in evergreens might act as priority storage sites, i.e. they contain more stored NSC or
186 N than predicted from their size (Uscola et al 2015). Specific adaptive traits can modify this
187 general trend. Thus, root or stem resprouters generally allocate more C and N to the roots or
188 stems, respectively, than do non-sprouting species (Palacio et al. 2007).

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190 **3) The contribution of stored NSC and N to new root and shoot growth**
191 New root growth immediately after planting is crucial to hydrologically couple seedlings to the
192 soil (Grossnickle 2005). Poor initial root growth can cause seedling water stress that hinders
193 photosynthesis and growth, ultimately leading to damage or mortality. This process and other
194 physiological processes involved in seedling establishment were synthesized in Burdett's
195 plantation establishment model (Burdett 1990). In seasonally dry climates, such as the
196 Mediterranean and Tropical climate, the main challenge for seedlings after establishment
197 during the wet season is to survive the dry period. This is linked to the capacity of seedlings
198 to avoid lethal water stress, which is mostly achieved by having a large, deep root system
199 during the dry season (Grossnickle 2005; Padilla and Pugnaire 2007; Villar-Salvador et al.
200 2012). Because growth is very sensitive to reduction in plant water potential (Hsiao 1973),
201 seedling root elongation will remain arrested during the dry period (Padilla and Pugnaire
202 2007) as long as the plant is under water stress. Consequently, seedlings need to produce a
203 large and deep root system before the onset of the dry period. Furthermore, planted

204   seedlings produce new shoots prior to the dry season, which usually enhances plant

205   photosynthetic and competitive capacity (Cuesta et al 2010).

206   Burdett (1990) suggested that current photosynthesis largely fuels root elongation

207   during seedling establishment. This is the dominant paradigm mainly for conifers among

208   forest managers and scientists (Luxmoore et al. 1995; Ritchie and Landis 2010). The

209   paradigm stems from studies published in the 1970-90s, which showed that shading, girdling

210   or growing plants in CO_2-depleted atmospheres depresses root growth in conifers (van den

211   Driessche 1987; Philipson 1988; van Den Driessche 1991b; Noland et al 1997). Similarly, ^{14}C

212   or ^{13}C pulse labeling studies, which only allow quantifying partitioning of labeled

213   photosynthates throughout the plant also seemed to support this paradigm (Gordon and

214   Larson 1968; Ursino et al. 1968). For instance, van den Driessche (1987) observed that the

215   ^{14}C recovered in new roots during the spring in ^{14}C-fall labeled plants, was comparatively

216   lower than the ^{14}C recovered in seedlings that were labeled during active spring root growth.

217   All these studies point out that current photosynthesis is intricately involved in new root

218   growth of conifers but they do not indicate the magnitude of its contribution to final

219   construction of new roots.

220   Stored NSC can also significantly contribute to new organ growth, independently if the

221   plant remains photosynthetically active. For instance, in two broadleaf evergreen trees and

222   one evergreen conifer, stored C made up > 65% of total C in new fine roots during early

223   spring (Uscola et al. 2015). However, for deciduous trees in which new roots resume

224   elongation before budburst (Riedacker 1976), initial new root and shoot growth relies

225   completely on stored NSC (Dyckmans and Flessa 2002; Vizoso et al 2008).

226   Other stored resources along with NSC must be considered to control root and shoot

227   growth during seedling establishment and the subsequent rapid growth phase in spring.

228   Thus, in the evergreen conifer *Pinus sylvestris*, remobilization of N, K and Mg supplied 83, 82

229   and 52%, respectively of new shoot growth demand of these nutrients (Proe et al. 2000).

230   Similarly, remobilized N contributed to early root growth in four evergreen species more than

231   current uptake of soil N (Uscola et al. 2015).

232   We quantitatively reviewed published studies (see Supplementary material for

233   methodological details of the review and consulted studies) to assess if main functional types

234   of woody plants (conifers, and broadleaves both evergreens and deciduous) differ in the use

235   of stored NSC and N to the construction of new shoots and roots. Regarding N, the

236   contribution of storage to new shoot growth decreased through time in conifers and

237   deciduous broadleaf species, while it increased among evergreen broadleaf species (Figure

238   1). New shoots/leaves were mostly made of remobilized N in conifers and deciduous

239   broadleaf species (> 65%, 1 and 2 months after bud break) with small differences between

240   both functional types. The proportion of remobilized N in new shoots/leaves of evergreen

241   broadleaves was significantly lower (< 50%) than in conifers and broadleaf deciduous

242   species 1 and 2 months after budburst. Regarding C, we did not detect any effect of time

243   after budburst on the composition of organs in remobilized C. The proportion of remobilized

244   C in new leaves/shoots was on average lower than 50% in all functional types, without

245   significant differences among them (Figure 1). The low dependence on remobilized C for

246   new shoot/leaves in broadleaf deciduous woody plants may provide an erroneous

247   perspective of the storage physiology of these plants as most data in the database were

248   taken when foliage was nearly or completely unfolded. Deciduous trees can allocate high

249   amounts of currently fixed C to new organ growth after reaching autotrophy, which usually

250   occurs rapidly after budburst and significantly before new leaves are completely mature (Keel

251   and Schädel 2010). However, during budburst and in the very early shoot growth stages,

252   deciduous plants mainly use stored C to support leaf/shoot growth (Dyckmans & Flessa

253   2002).

254   In conifers, contribution of stored N and C to the construction of new fine roots was low

255   while in deciduous woody plants the contribution of remobilized N was high (Table 2). The

256   proportion of remobilized N in new fine roots of broadleaf evergreens was low and similar to

257   values in conifers. Conversely, remobilized C in new roots of broadleaf evergreen species

258   was higher than in conifers and similar to values in broadleaf deciduous species. Notably, the

259   limited data on conifer new fine roots (*Pinus nigra* and *Pinus halepensis;* Maillard et al. 2004;

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260   Uscola et al. 2015) seem to support the idea that conifers strongly rely on current

261   photosynthesis and soil N for early new root growth. We found very few studies reporting the

262   contribution of C and N reserves to new (fine) root growth, which probably precluded finding

263   significant differences among plant functional types (Table 2). Therefore, results should be

264   taken with caution. The low number of species in our data base was because most of these

265   studies did not distinguish between fine and coarse roots. Both types of roots should be

266   considered separately because they have distinct sink strength for remobilized resources

267   (Uscola et al. 2015) and varying patterns of nutrient uptake (Hawkins et al. 2014).

268   Our quantitative review indicates that new fine roots tend to depend more on external

269   sources for their construction in spring while new leaves/shoots tend to be constructed using

270   more remobilized resources than external resources, especially for N (>50%) (Figure 1 and

271   Table 2). This might be explained because new leaf/shoot growth is a stronger N and NSC

272   sinks than new root growth (Grelet et al 2001; Uscola et al. 2015), which likely results in

273   external resource uptake not meeting resource demand for new leaf/shoot growth.

274   Differences in the contribution of remobilized resources in the construction of new roots and

275   new leaves/shoots has important implications for seedling cultivation, highlighting the

276   importance of N- and NSC-loading seedlings for fueling shoot growth after seedling

277   establishment.

278   Dependence of new root growth on stored C in broadleaf deciduous woody plants will

279   presumably depend on whether root elongation resumption is before or after foliage

280   development. When shoot growth occurs before new fine root elongation due to low soil

281   temperature, new root growth can be supported by C supplied by new foliage. Similarly,

282   when soil N take up does not meet plant N demand due to either low soil temperature or low

283   N availability, new growth strongly relies on remobilized N (Millard and Proe 1993; Millard et

284   al. 2001; Silla and Escudero 2003).

285   The role of plant organs as suppliers of remobilized resources differed among woody

286   plants. In (evergreen) conifers, most of the N remobilized to support new organ growth of

287   juveniles is derived from foliage (Table 3). Consequently, stems and roots in conifers seem

to be poor sources of remobilized N. Conversely, stems and roots supplied 50% of remobilized N in broadleaf evergreens. Finally, stems contributed slightly more N to remobilization than roots in broadleaf deciduous woody plants. Interestingly, we observed that the contribution of an organ to N remobilization is directly related to the contribution of the organ to the seedling´s initial N content (Figure 2). However, leaves in evergreens (both broadleaf and conifers), and stems to a lesser extent, proportionally "donate" more remobilized N than predicted by their contribution to seedling N content. In contrast, roots donate less N than predicted by their contribution to seedling stored N. This is implicated by the fact that most foliage and stem data in Figure 2 have a proportion of remobilized N relative to their contribution in seedling N content > 1 (i.e., data are above the line that represents equal proportion of remobilized N and contribution in seedling N content), while this proportion is < 1 for most root data. Two causations may help to explain this trend. First, it is possible that seedlings follow a resource conservation strategy and may only have a greater use of the N stored in roots when recovery after disturbances or other stress factor that require a surplus of N. Second, the proportion of soluble N (mobile N) might differ among organs types (Vizoso et al. 2008).

Data on the contribution of plant organs to remobilized C during new shoot and root growth in spring at a whole plant scale are scarce. In three Mediterranean broadleaf evergreen trees, Uscola et al. (2015) found that old leaves (45-59%) and coarse roots (25-34%) were the main contributors of remobilized + respired C during spring growth. In the Mediterranean pine, *P. halepensis*, needles supplied around 73% of lost C during spring growth (Uscola et al. 2015). In contrast, *Pinus nigra* foliage had a minor role and roots supplied 75% of lost C (Maillard et al. 2004). Finally, in the seedlings of the deciduous tree *Quercus robur,* stems and coarse roots contributed 46 and 54% to the C remobilized and respired for spring growth (Vizoso et al. 2008).

Remobilization of stored NSC and N for spring growth usually reduces their concentration over the short term. However, reduction of N reserves often is greater than NSC reserves (Millard & Grelet 2010), which recover faster than N reserves after spring

316   growth (Bazot et al. 2013). Seedling establishment seems to have a low impact on seedling

317   stored NSC. In three out of five Mediterranean trees species, NSC concentration in 1-year-

318   old seedlings had a clear increase in NSC relative to pre-planting NSC values when

319   seedlings were outplanted under full sun conditions for 26 days (Figure 3). In the oak *Q. ilex*,

320   NSC had no variation while NSC significantly decreased relative to pre-planting NSC values

321   in *Q. coccifera*. Under moderate stress conditions (i.e., reduction in photosynthesis due to

322   65% reduction in photosynthetic photon flux density), NSC pools were little affected in all

323   species except for *Q. coccifera*, which showed a strong reduction. Thus, under non-limiting

324   or moderate stress field conditions current photosynthesis usually replenishes seedling NSC

325   pools rapidly during the establishment phase. Finally, only when current photosynthesis was

326   suppressed by shoot darkening, did NSC pools strongly decline in all species, but primarily in

327   the two fast growing pines. Shoot growth in spring has a greater impact on seedling stored

328   resources than new root growth during the establishment phase because it is a stronger

329   resource sink than early new root growth (Uscola et al. 2015). In 1-year-old *Q. robur*

330   seedlings, woody compartments (the main sources of remobilized N and C) had a net loss of

331   N after spring shoot growth and new N uptake could not replenish initial storage levels on the

332   short term. In contrast, C content of woody compartments recovered in the same period to

333   the pregrowth levels as a result of currently assimilated C (Figure 3). Thus, contrary to N,

334   plants rarely are C-limited in most growth conditions and C storage is maintained even when

335   subjected to a short growing season (Hoch and Körner 2003) or to repeated cutting or

336   grazing (McPherson and Williams 1998b; Palacio et al. 2008; Palacio et al. 2011).

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338   **4) Factors affecting resource mobilization**

339   As with other functional attributes, mobilization of stored resources shows plasticity and

340   varies depending on environmental and plant endogenous factors. Most published literature

341   on the environmental factors affecting mobilization of stored compounds has been performed

342   with N, while mobilization of NSC has received little attention. As shown in the previous

343   section, the use of remobilized compounds for new growth varies among plant functional

types and across the growing season (Figure 1), among years (Silla and Escudero 2003) and changes through ontogeny (Gilson et al. 2014). Uscola et al (2015) also found that the use of remobilized N and C for new shoot growth increased with relative growth rate among evergreen woody plants. In the following section we review other factors that are relevant for seedling outplanting performance and that can affect the way plants use stored resources.

4.1 Plant N content

Nitrogen remobilization is mainly a source-driven process, i.e., the greater the amount of stored N, the higher N remobilization is, which increases growth capacity (Millard 1996; Millard and Grelet 2010). This, in part, explains why high N content plants have superior growth and are more competitive after transplanting in the field than are low N plants (van den Driessche 1991a; Cuesta et al 2010; Villar-Salvador et al 2013b; Oliet et al 2013a). Conversely, NSC remobilization is a sink strength driven process and consequently the amount of remobilized NSC does not depend on the size of NSC storage (Millard and Grelet 2010).

Demonstrating that increasing N remobilization enhances new growth is not an easy task. Remobilization experiments frequently have confounding effects that complicate the ability to identify specific factors that control plant growth. For instance, nursery N fertilization increases seedling N content (see section 6) and bud development (Islam et al. 2009) and these plants can have higher outplanting growth (Villar-Salvador et al 2013). To ascribe this superior growth to remobilization may be erroneous because an increase in plant N content often co-varies with photosynthetic capacity in N fertilization experiments: high N fertilized seedlings have greater foliage with a higher photosynthetic rate than do low N fertilized plants (Oliet et al. 2013), which can also drive outplanting growth (Guehl et al. 1989). We designed a study to control for these confounding effects and to assess if differences in N remobilization are related to growth differences. We produced two phenotypes of *P. halepensis* seedlings, which had the same size with no differences in morphology but differed strongly in their N content (Table 4). This was achieved by using a fall fertilization

371 regime where half of the plants were supplied with a high N fertilization rate under cool

372 temperature conditions, which prevented plants from growing but did not completely hinder N

373 uptake capacity. The remaining plants were not fertilized during the fall. Seedlings were

374 transplanted to a plot in early winter and subjected to strong weed competition in spring.

375 Further cultivation and field measurement details can be consulted in the legend of Table 4

376 and in Cuesta et al. (2010). Both types of seedlings did not differ in photosynthesis rate in the

377 nursery before transplanting. However, spring photosynthesis rate measured under field

378 conditions was significantly lower in the fall N-loaded plants than in the unfertilized plants. At

379 the end of the spring, shoot growth was 60% higher in N-loaded plants than in seedlings that

380 were not fertilized in the fall. Concomitantly, N remobilization was 3.5 times higher in fall N

381 loaded plants than in unfertilized N seedlings, while soil N uptake did not differ among

382 seedling types. These results supports the idea that greater remobilization due to higher N

383 content enhances field growth.

384 *4.2 Soil fertility*

385 Performance on poor soils can be increased if planted seedlings have high nutrient use

386 efficiency. It has long been suggested that this could be attained if plants have high

387 resorption efficiency and mainly rely on stored resources for new growth, which would reduce

388 dependence on soil nutrients (Chapin III 1980; Salifu and Timmer 2003a). However,

389 evidences indicate that N remobilization seems to be unaffected or little by soil fertility across

390 plant functional types (Millard and Neilsen 1989; Millard and Proe 1991; Proe et al. 2000;

391 Grelet et al. 2001; Cheng and Fuchigami 2002; Salifu and Timmer 2003a). Similarly, Aerts

392 (1996) reviewed resorption efficiency studies covering 60 woody species and concluded that

393 63% of the species showed no response to soil fertility while 32% of the species showed a

394 decrease in N resorption efficiency.

395 *4.3 Competition and herbivory*

396 Planted seedlings often are predated to varying degrees and have to compete with

397 established vegetation. At a within-species scale, stored resources can play a significant role

398   in overcoming browsing, disease and competition (McPherson and Williams 1998a; Cruz et

399   al 2003; Cuesta et al 2010). Stored C rather than current photosynthesis provided most of

400   the C used for the construction of reaction tissues in *P. sylvestris* infected with a pathogenic

401   fungus (Guérard et al. 2007).

402   Competition hinders growth and thus future competition capacity of plants by reducing

403   the amount of stored resources. In *Betula pubescens*, competition resulted in smaller

404   seedlings with less ability to store and, consequently, to remobilize N in the next growing

405   season (Millet et al. 2005). However, competition did not hinder current N remobilization, but

406   it reduced N uptake in *P. halepensis.* Consequently, remobilized N was the main source for

407   new spring growth in seedlings competing with weeds, while soil N was the main N source in

408   seedlings growing without weeds (Cuesta et al 2010).

409   Damage of shoots frequently results in compensatory growth by increasing

410   photosynthesis of undisturbed leaves and mobilization of stored resources in intact organs

411   (Canadell & Lopez-Soria 1998; Oyarzabal & Oesterheld 2009). However, as for competition,

412   herbivory may also reduce future remobilization capacity after a portion of stored resources

413   are removed by predators (Millet et al. 2005). This explains in part why removal of the old

414   leaves before bud burst in *Q. ilex* reduced N concentration in new leaves grown in spring

415   (Cherbuy et al. 2001). The capacity of plants to use stored resources to recover after

416   predation depends on the main storage sites. Among woody species, leaf predation may

417   have a greater impact on evergreen species than in deciduous species because leaves are

418   the main N storage sites in evergreens while woody organs are the main N storage sites in

419   deciduous species (Millard et al. 2001). Accordingly, it can be hypothesized that conifers

420   should be more vulnerable to leaf predation than broadleaf evergreen species (Table 3).

421   *4.4 Water stress and shade*

422   Few studies have analyzed the effect of water stress or shade on remobilization. As for

423   competition, drought stress or shading in the current growing season may hinder

424   remobilization capacity in the next growing season if it decreases seedling N and NSC

425   content. Reduction in light to 20% reduced starch content 50% in *Vitis vinifera roots*, which

426   are main sites of remobilized resources for spring growth. Consequently, leaf and roots were

427   smaller in the next growing season compared with non-shaded vines (McArtney and Ferree

428   1999).

429   In contrast to remobilization, the effect of drought on foliage nutrient resorption has

430   been studied more thoroughly. Premature and non-programmed leaf abscission in response

431   to water stress may result in low nutrient resorption (del Arco et al. 1991; Harvey and van

432   den Driessche 1999) with potential consequences for N storage and future remobilization.

433   However, drought-induced leaf abscission will not affect N storage and growth the following

434   year, provided that N uptake can occur during the cold season (Wendler and Millard 1996).

435

436   **5) The role of NSC and N reserves in seedling drought and frost resistance**

437   Plants seldom are C-limited but seedlings can be subjected to conditions between the

438   nursery and the plantation site that significantly reduce seedling NSC and, consequently,

439   may jeopardize out-planting performance. For instance, seedlings stored in darkness in

440   refrigerated systems may have lower outplanting performance if not adequately hardened or

441   stored for extended periods of time (Grossnickle and South 2014). Poor field performance of

442   cold stored plants has been attributed in part to strong reduction in NSC levels (Ritchie 1982,

443   Table 5). However, few studies have found a close relationship between field performance

444   and seedling NSC following storage (Puttonen 1986; Cannell et al. 1990; Omi et al. 1994).

445   Similarly, seedlings exposed to prolonged drought stress either after spring shoot growth or

446   when planted under deep shade, such as in the understory of forest stands, might also show

447   severe reductions in tissue NSC levels (O'Brien et al. 2014).

448   The capacity of planted seedlings to withstand frost and drought is related to tissue

449   NSC concentration, mainly of soluble sugars (Sakai and Larcher 1987; Morin et al. 2007).

450   Perennial plants from temperate and boreal biomes become increasingly frost tolerant

451   through the fall and winter (Sakai and Larcher 1987; Mollá et al 2006). This concomitantly

452   occurs in parallel to an increase in tissue soluble sugars concentration (Ögren et al. 1997;

453   Heredia et al. 2014). Starch hydrolysis provides a significant part of soluble sugars during

454 cold hardening, which stabilizes cell membranes against cell dehydration caused by the

455 freeze of extracellular water (Levitt 1980; Uemura and Steponkus 2003). Premature

456 reduction in cold hardiness due to higher NSC respiratory consumption has been reported

457 during spells of mild winter temperatures (Ögren et al. 1997). Galvez et al. (2013) observed

458 that prolonged drought stress in the previous growing season enhanced winter mortality in

459 two *Populus* species. This was attributed to low frost tolerance of root system linked to

460 reduced root NSC. Similarly, *Q. ilex* seedlings cultivated in a mild winter nursery had 32%

461 less soluble sugar concentration and higher shoot dieback after heavy frosts in the field than

462 seedlings cultivated in a cold winter nursery (Mollá et al. 2006).

463   The relationship between frost hardiness and seedling N storage is less straightforward

464 than with NSC. There is general agreement that low tissue N concentration usually hinders

465 cold acclimation (van den Driessche 1991a; Taulavuori et al. 2014). However, moderate to

466 high tissue N concentration has been shown to increase (Andivia et al. 2012), reduce

467 (Harvey and van den Driessche 1997) or have no effect on frost resistance (Thomas and

468 Ahlers 1999). Taulavuori et al (2014) reviewed 50 studies published since 1990 to 2014 and

469 found that in 40% of cases higher N supply increased frost hardiness, while in 29% of cases

470 N had no effect. They concluded that plant frost hardiness generally increases when N

471 increases within the optimal range for a given species. High tissue N concentration can

472 increase frost tolerance by increasing NSC, as a result of enhanced photosynthesis, and the

473 concentration of proteins involved in frost tolerance (Sheppard 1994; Duman and Wisniewski

474 2014). However, high tissue N concentration can also hinder frost hardiness if it delays shoot

475 elongation cessation in the fall (Heredia et al. 2014).

476   As per cold tolerance, poor performance under dry conditions at an intraspecific level

477 has also been linked to lower plant NSC. McDowell et al. (2008) proposed C starvation to be

478 an important driver of tree mortality under dry conditions and several studies have provided

479 indirect support for this idea. For instance, a large decrease in tissue NSC concentration at

480 planting due to prolonged storage in refrigerated systems increased mortality and reduced

481 growth after planting in *P. sylvestris* seedlings (Figure 4). The relation was not linear,

17

482 indicating that deterioration of plant performance does not occur until a critical NSC threshold

483 is achieved. Similarly, dieback in *P. sylvestris* adults after prolonged and intense drought in

484 NE Spain was greater in individuals that had the lowest NSC concentration (Galiano et al.

485 2011). Prolonged and intense drought reduced root and needle starch concentration in *P.*

486 *nigra* seedlings, with mortality increasing rapidly when plants reached very low tissue starch

487 levels (Guehl et al. 1993). However, these experiments provide circumstantial evidence

488 regarding the role of NSC on plant mortality. Demonstrating that low stored NSC is a cause

489 of plant mortality is a very difficult task due to many confounding effects. Recent experiments

490 that were designed to specifically unravel the role of NSC on plant survival, have provided

491 solid evidences that low NSC can jeopardize the capacity of plants to survive under dry

492 conditions (Mitchell et al. 2013; Hartmann et al. 2013; O'Brien et al. 2014). For instance, by

493 experimentally manipulating light conditions, O'Brien et al. (2014) produced seedlings with

494 contrasting NSC concentration in 10 Borneo tree species without differences in morphology.

495 After transplanting under drought conditions they observed that NSC enriched seedlings had

496 delayed mortality and maintained higher water potential and NSC levels than low NSC

497 seedlings.

498 NSCs are involved in osmotic adjustment, embolism and oxidative stress repair and

499 defense, which are crucial physiological processes for plants to survive drought (Chaves et

500 al. 2003; Salleo et al. 2004; Sevanto et al. 2014). Therefore, strong reduction in NSC can

501 constrain the capacity of plants to meet NSC demand for these physiological processes and

502 for maintenance metabolism (McDowell 2011). Moreover, water stressed plants have limited

503 capacity to translocate stored NSC due to phloem failure, limiting NSC transport to where

504 needed (Hartmann et al. 2013; Sevanto et al. 2014).

505 The importance of low NSC as a cause of seedling mortality depends on the strategy of

506 plants to resist drought (McDowell et al. 2008; Mitchell et al. 2013). Drought avoiders or

507 isohydric species, which close stomata at high tissue water potential, are prone to mortality

508 through NSC-based processes as they potentially can be exposed to sustained periods of

509 negative carbon balance. In contrast, drought tolerant or anisohydric species are able to

510   maintain gas-exchange capacity at low water potential values, which prevents significant

511   NSC reductions during dry periods. For anisohidric plants hydraulic failure caused by xylem

512   embolism seems to be the main cause of death (McDowell et al. 2008; Mitchell et al. 2013).

513   Low seedling survival under dry conditions has also been related to low tissue N

514   concentration (van den Driessche 1988; Oliet et al. 2013). Higher tissue N can support

515   higher root and shoot growth as discussed in Section 4. This is important for seedling

516   establishment and vigorous root growth before the onset of the dry season, which is crucial

517   for maintaining water uptake capacity during the dry season and gas-exchange capacity

518   (Villar-Salvador et al. 2012).

519

520   **6) Nursery cultivation practices affecting seedling NSC and N storage**

521   Seedling quality is strongly influenced by nursery cultivation practices such as irrigation,

522   fertilization, hardening, cold storage, and lifting/store or handling (van den Driessche 1991b;

523   McKay 1994; Wilson and Jacobs 2006; Islam et al 2009; Grossnickle 2012; Grossnickle and

524   South 2014). These manipulations may result in changes in seedling size, root/shoot

525   biomass allocation, cold hardiness, dormancy development, stress tolerance and storage

526   reserves that affect subsequent seedling outplanting performance (Grossnickle 2012).

527   Specifically, understanding how seedlings use stored resources to support growth is of

528   practical importance for cultivating seedlings. Nursery practices should promote NSC and N

529   storage in those species that strongly depend on these stored reserves for new growth. This

530   can be accomplished by optimizing fertilization systems. Similarly, nurseries should promote

531   traits conferring high C assimilation and soil N acquisition in species that primarily use

532   external C and N sources to support new growth. This can be achieved by producing

533   seedlings with high leaf area, photosynthetic rate, and large root systems. Photosynthetic

534   rates can usually be enhanced by increasing foliar N concentration through an optimized

535   fertilization regime.

536   Storage of NSC and N can be quantified using two components that although related,

537   have different consequences for plant function. Compound "concentration" is relevant for

538   osmotic- and mass flow rate-based physiological processes such as osmotic adjustment and

539   cell membrane stability, cold hardiness and gas-exchange (Chaves et al. 2003; Villar-

540   Salvador et al.2004b; Warren and Adams 2004; Morin et al. 2007), while compound "content"

541   better explains remobilization and absolute growth, persistence under sustained negative C

542   balance situations or disturbance recovery (Poorter and Kitajima 2007; Millard and Grelet

543   2010; McPherson and Williams 1998). Thus, if two plants of the same species have the

544   same N and NSC concentration but differing content of these compounds (for instance

545   because one is larger than the other) and stored compounds are equally partitioned, both

546   plants would likely have the same frost tolerance and photosynthetic rate but the plant with

547   higher N and NSC content will have greater remobilization, growth capacity, and potential to

548   persist under deep shade conditions. A summary of the primary nursery cultivation practices

549   and their effects on seedling NSC and N concentration and content is shown in Table 5.

550   While culture is generally designed to minimize resource limitations and promote seedling

551   growth, applying some level of stress to slow growth (and its corresponding demand for

552   nutrients and current photosynthates) or supplying resources at higher rate than seedling

553   growth rate may cause plants to re-allocate these resources toward reserves (Chapin III et al

554   1990; Grossnickle et al. 1991; Landhäusser et al 2012a). The final effect on NSC and N

555   seedling content will depend on the balance between growth and concentration of these

556   compounds.

557   Four aspects of **fertilization** can be modified to change seedling nutritional status:

558   dosage, form of application, timing of application and N form applied. *Dosage*: N-based

559   fertilizers (e.g., urea or ammonium nitrate) generally increase N concentration and content

560   although the magnitude of response is species-specific and dependent on fertilizer

561   application method (Birge et al 2006; Villar-Salvador et al 2013b). Conventional fertilization

562   practices in bareroot and container seedling nurseries are often based upon providing

563   seedlings with a constant fertilizer supply throughout the growing season using a moderate

564   fertilizer supply (Landis et al 1989). While use of this method usually achieves seedling

565   morphology targets, there is a tendency for decline in foliar nutrient concentration due to

growth dilution as the season progresses, which suggests nutrient stress due to excess fertilization at the beginning of the growing period and possible under-fertilization at the end of culture (Timmer 1997). Increasing the amount of nutrients applied can enhance formation of reserves but can extend the growing period and decrease the hardening of the seedlings, and thus reduce the cold resistance of the seedlings (Islam et al. 2009; Andivia et al. 2011). Effects of fertilization methods on NSC are less well studied though results generally suggest that fertilization decreases NSC concentration (Sanz-Pérez et al. 2007; Villar-Salvador et al. 2013). Because fertilization also stimulates growth the final effect on plant NSC content will depend on the balance between NSC concentration and plant mass.

Form of application: While fertilization is generally targeted to root systems by application to soil or media, foliar fertilization represents an alternative application method to increase N reserves (Montville et al 1996; Uscola et al 2014; Wuyts et al 2015), especially if applied in late summer and in the fall.

Modifying the *time of application* can help rectify some of the disadvantages of increasing nutrient dosage. In this sense, exponential fertilization tailors fertilizer inputs to plant demand, generally involving low fertilization at the beginning of culture and high fertilization during the final stages of cultivation (Timmer and Aidelbaum 1996; Timmer 1997). This allows for luxury nutrient consumption, with optimal fertilization being that which maximizes N content without inducing toxicity (Salifu and Timmer 2003b; Salifu and Jacobs 2006; Birge et al. 2006) and produces N-loaded seedlings. Late season (fall) fertilization is a variant of N loading whereby conventionally grown seedlings are fertilized at relatively high rates after bud set in fall (van den Driessche 1985; Islam et al. 2009; Oliet et al. 2011; Andivia et al. 2014), which contrasts with traditional hardening practices that imply little to no fertilization during this period (Landis et al. 1989). However, even though this fertilization technique can enhance N concentration and content, the effect on NSC is not well known. For instance, Heredia et al (2014) did not find leaf soluble carbohydrate concentration differences among conventional, exponential and fall fertilization in *Q. ilex*.

593   Finally, *N form* used in the fertilization program has a strong impact on physio-
594   morphological attributes of seedlings. NH_4^+ fertilization tends to hinder plant growth,
595   especially of fine roots, increasing plant N concentration, however it can reduce
596   concentrations of other cations in plants and cause toxicity when applied at high rates (Britto
597   and Kronzucker 2002; Uscola et al 2013). In contrast to NH_4^+, NO_3^- can be commonly
598   supplied at higher concentrations without harmful effects on plants but easily stimulates
599   growth even later in the growing season by diluting the N concentration of the seedlings
600   (Landis et al 1989; Cruz et al 1993). Some nurseries alter the primary N form in the fertilizer
601   depending on the season, using mainly NO_3^- during the spring and NH_4^+ in late season to
602   stop growth and increase N concentration of the seedlings (Landis et al. 1989).

603   **Irrigation** intensity is usually adjusted according to growth phase and plant needs, with
604   inputs gradually increasing during the growing period and a withholding of watering at the
605   end of culture to help induce hardening (Landis et al. 1989). Reduced irrigation throughout
606   the duration of culture results in lower plant biomass and less reserves (Timmer and Miller
607   1991). However, moderate stress applied for short periods during the hardening phase
608   usually increases N and increases or has no effect on NSC concentration, but intense and
609   prolonged drought stress act to decrease NSC concentration (Guehl et al 1993; Stewart and
610   Leiffers 1993; McDowell 2011; Villar-Salvador et al. 2004b, 2013a), eventually leading to
611   mortality. The threshold balance between positive and negative effects of drought hardening
612   on seedling development and its relation to N or NSC reserves are largely unstudied and
613   provide opportunity for future research (Villar-Salvador et al. 2004b, 2013a). Evidence
614   suggests that there may also be interactive effects of drought hardening and fertilizer
615   application method, as Timmer and Miller (1991) reported increased N concentration in roots
616   under dry conditions for exponential vs. conventional fertilization and the opposite effect
617   under a moist irrigation regime.

618   Other cultural practices that influence N and NSC reserves include **shading**, which
619   generally decreases both compounds when applied for extended duration through the
620   growing season (Valladares et al. 2000; Villar-Salvador et al. 2004a; Baber et al. 2014).

621   **Photoperiod manipulation** (i.e., blackout or short-day treatment) involves reduction of

622   photoperiod for several weeks during mid-summer and is often applied to species in boreal

623   zones as a means to slow shoot growth and induce hardening. While researchers have

624   emphasized dormancy and cold hardiness responses, evidence suggests that this practice

625   may result in an increase or essentially no effect on N or NSC concentration (Grossnickle et

626   al. 1991; Tan 2007; Landhäusser et al. 2012). **Cultivation density** (bareroot and container)

627   or **container volume** also influence reserves, with generally an increase or no effect on N

628   concentration and content as density decreases or container volume increases (van den

629   Driessche 1984; Robbins and Pharr 1988; Aphalo and Rikala 2003; Dominguez-Lerena et al

630   2006; Oliet et al 2012; Dumroese et al 2013); effects on NSC are less well documented,

631   though a general trend of increasing seedling dry mass as density decreases or as container

632   volume increases suggests that NSC reserves would increase concomitantly. Winter storage

633   conditions may also affect N or NSC reserves. In Mediterranean environments, seedlings are

634   generally stored outdoors until being transported for planting; exposure to lower fall and

635   winter temperatures may result in accumulation of greater N and NSC reserves than that in

636   warmer sites for the same genetic source (Mollá et al 2006; Heredia et al 2014). In temperate

637   and boreal enviroments, seedlings are often stored in coolers (above or below freezing);

638   prolonged cold storage acts to decrease NSC reserves (Ritchie 1982; Puttonen 1986;

639   Cannell et al 1990). Finally, poor **handling** practices can have a profound negative effect on

640   NSC reserves; for example, Insley and Buckley (1985) reported that losses of lateral roots of

641   broadleaved seedlings during plant handling might account for 20–40% of total root

642   carbohydrates.

643

644   **7) Future research directions**

645   Many studies have addressed the role of N remobilization on plant growth (see

646   Supplementary material). However, most of these studies have been performed with

647   temperate and boreal trees. Little information exists on species of other biomes and other

648   functional types of woody plants, such as shrubs and chamaephytes (but see Palacios et al.

649   2014; Uscola et al. 2015). The need for studies on the use of NSC reserves for new growth is

650   even more peremptory than stored N as we cannot presently elucidate clear use patterns of

651   remobilized NSC among woody plants. Moreover, most studies have been performed on

652   single species and studies have been made under different growth conditions, making

653   conclusions on the adaptive meaning of stored resources use patterns difficult. Therefore, we

654   need more comparative studies performed under the same environmental conditions (see

655   Millard et al. 2001; Uscola et al. 2005).

656   Most studies showing a relation between plant survival, growth and disturbance

657   recovery and NSC levels provide circumstantial evidence of the role of stored NSC in plant

658   performance. Several confounding effects make it difficult to experimentally demonstrate the

659   role of NSC on drought and cold survival. For instance, NSC content has been related to

660   disturbance recovery of plants. However, it is possible that other resources such as N and P

661   may co-vary with NSC and be as relevant as NSC for plant recovery (Moreira et al 2012).

662   Therefore, a major challenge of future research is designing experiments to address the

663   specific role of major stored resources and their interactions.

664   Nitrogen nutrition affects the frost tolerance of plants but there is no clear consensus as

665   to whether moderate to high N supply during the cold acclimation period increases frost

666   tolerance in plants. The varying effects of nutrition on plant cold and drought tolerance might

667   be due to differences among studies in whether seedlings were evaluated during active

668   growth or in dormancy, species-specific differences and due to interactions with several

669   environmental factors such as temperature during the cold acclimation phase. Therefore for

670   late season fertilization be an effective seedling N-loading option, future research should

671   assess which species are most suitable for this fertilization procedure and the optimum

672   period during fall when it could be applied without negative effects on cold hardening.

673   Finally, there are several cultivation practices for which their effect on concentration

674   and content of NSC are unknown such as fall and foliar fertilization, cultivation density and

675   container volume.

676

8) Conclusions

Juveniles across functional types of woody plants show important differences in how they store and manage N and NSC reserves for spring growth and stress tolerance. Overall, broadleaf deciduous seedlings accumulate more N and NSC in roots than do broadleaf evergreens and conifers. *Quercus* species partitioned more stored resources to roots than the non-*Quercus* species. Among evergreens, conifers showed the highest N partitioning to leaves. In general, woody plants tended to use more remobilized resources for the construction of new leaves/shoots than for the new fine roots protruding out of the root plug after planting. However, conifers used less remobilized resources than broadleaf species for new fine root growth. Spring new shoot/leaf growth was mostly made of remobilized N (>60%) in conifers and broadleaf deciduous species, while broadleaf evergreens relied more on soil N. In contrast, the contribution of remobilized C to new leaves/shoots showed few differences among functional types and comprised 28 to 45% of organ C. This highlights the importance of current photosynthesis for new leaf/shoot growth and the differences between C and N remobilization physiology. The organs that supplied more remobilized N to support growth had higher contribution to seedling N content. However, leaves in evergreens seem to supply more remobilized N than predicted by their contribution to seedling N content, while roots showed the reverse pattern. Under non-limiting conditions, NSC storage is little affected after spring growth as current photosynthesis rapidly replenishes NSC storage. However, when seedling photosynthesis is depressed for long periods such as in cool storage, or due to intense and sustained drought stress or deep shade, NSC reserves can be significantly reduced, which potentially can impair outplanting performance. In contrast, N reserves usually are significantly reduced after seedling establishment or during early spring growth and their replenishment takes longer than NSC storage. Cultivation practices that apply some level of stress to slow growth of seedlings and supply resources at a higher rate than that of seedling growth rate can promote resource storage.

9) Acknowledgments

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10) References

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Figure legends

Figure 1. Contribution of remobilized N (A) and C (B) to new shoot/leaf growth in juveniles of

main functional types of woody plants. Data are means ± 1 SE. As the contribution of

remobilized N was influenced by the time after bud break (see text), subfigure A shows least

square means estimated at three moments after bud break. Subfigure B shows the

contribution of remobilized C after most leaves had completely unfolded and matured.

Inserted numbers are the number of species studied in each functional type. See

Supplementary material for methodological details of the review.

Figure 2. Relationship between the relative contribution of an organ to total remobilized N in

a plant and the relative contribution of the organ to plant content. Data are from 17 species

and for each species, we show data for foliage, stems and roots, except for deciduous

plants, which lacked leaves as data were obtained from dormant seedlings. The dotted line

indicates the 1:1 relationship between both variables. Black symbols are data of

evergreenswhile white symbols are the data of deciduous woody species. Dots, inverted

triangles and squares are data for leaves, stems and roots, respectively. We show the values

for a representative conifer (Pn; *Pinus nigra*), broadleaf evergreen shrub (Qc; *Quercus*

coccifera) and a broadleaf deciduous tree (Qr; *Quercus robur*).

Figure 3. A) Variation in the content of old C (left subfigure) and old N (right subfigure) in

woody organs (coarse roots and stems) of 1-year old *Quercus robur* seedlings before bud

break and after leaf growth completion in spring. The white bar shows the C incorporated

due to current photosynthesis or N taken up from the soil (redrawn from data in Vizoso et al.

2008). B). Relative variation in seedling NSC concentration relative to pre-planting seedling

NSC concentration in 1-year old seedlings of five Mediterranean woody species, which were

planted in the field in March for 26 days. n=4. Seedlings were planted under three light

conditions: full sun, shade (photosynthetic flux density was reduced by 65%) and complete

darkness. Shade was achieved by enclosing the shoots within an insect screen while

darkness was achieved by enclosing the shoots inside a polystyrene box wrapped with

aluminum foil. All seedlings grown under sun and shade conditions produced significant

amounts of new roots (Villar-Salvador, unpublished data). In both figures data are means ± 1

SE.

Figure 4. Relation of field mortality (black dots) and shoot growth (white dots) after one

growing season with the needle glucose concentration at planting in *Pinus sylvestris*

seedlings that were subjected to different storage regimes prior planting (adapted from

Puttonen 1986).

Table 1. Partitioning (%) of N and NSC in different organs in young plants (seedlings and saplings) in different functional types of woody plants. Data are from plants sampled in fall or winter prior to field planting and are means ± 1 SE. Last column shows statistical results of ANOVA or Kruskal Wallis tests. * P≤0.05; *** P≤0.001; see Supplementary material for literature review details and reviewed studies. Numbers in brackets indicate the number of species reviewed in a specific group.

	Broadleaf deciduous		Broadleaf evergreen			
	Non-Quercus	Quercus	Non-Quercus	Quercus	Conifers	
Nitrogen	(n=16)	(n=4)	(n=8)	(n=7)	(n=6)	
Leaves	0	0	37±3.5	40±3.8	55±4.1	H=35.6***
Stems	50±4.1	24±8.2	36±5.8	15±6.2	13±6.7	H=23.4***
Roots	50±3.8	76±7.7	31±4.3	46±5.8	32±4.6	F=8.9 ***
NSC	(n=9)	(n=3)	(n=6-11)	(n=6-7)	(n=8-12)	
Leaves	0	0	41±4.6	17±4.6	43±4.0	F=22.5***
Stems	39±3.5	18±6.1	30±4.3	14±4.3	26±3.8	F=5.77 **
Roots	61±4.2	82±7.3	29±3.8	72±4.8	28±3.7	F=26.8***

1 Table 2. Contribution (%) of remobilized N and C to the growth of new fine roots in three main

2 functional types of woody plants. Data are means ± 1 standard error and values in brackets are the

3 number of studied species in each group.

	Conifers	Evergreen broadleaf	Deciduous broadleaf
Remobilized N	15±4 (n=2)	20±5.5 (n=4)	66±26 (n=3)
Remobilized C	15±3.5 (n=2)	39±6 (n=4)	38±32 (n=2)

4

1

Table 3. Relative contribution (%) of different organs to remobilized N for seedling/sapling spring growth in three main functional types of woody plants. Data are means ± 1 standard error. See Supplementary material for methodological details of the review and used references.

	Conifers (n=4)	Broadleaf evergreen (n=5)	Broadleaf deciduous (n=8)	
Foliage	78±6	48±5	0	F=55.7 ***
Stems	7±5	30±4	54±3	F=32.8 ***
Roots	15±7	22±6	46±5	F=8.4 **

5

1 Table 4. Nursery functional attributes and outplanting performance differences between fall
2 unfertilized and fall N loaded *Pinus halepensis* seedlings. Plants were transplanted to a plot in early
3 winter and experienced very high weed competition in spring. Nitrogen remobilization and uptake,
4 and field net photosynthesis rate were measured at the end of the spring in five plants per
5 treatment. Remobilization and uptake were determined on the same plants used for gas exchange
6 measurements using a budget approach. Plant field growth was measured in late spring in 12-15
7 plants per treatment as the stem volume, which was calculated from the height and diameter of the
8 stem assuming a cone shape. Cultivation and further experimental details can be consulted in
9 Cuesta et al. (2010).

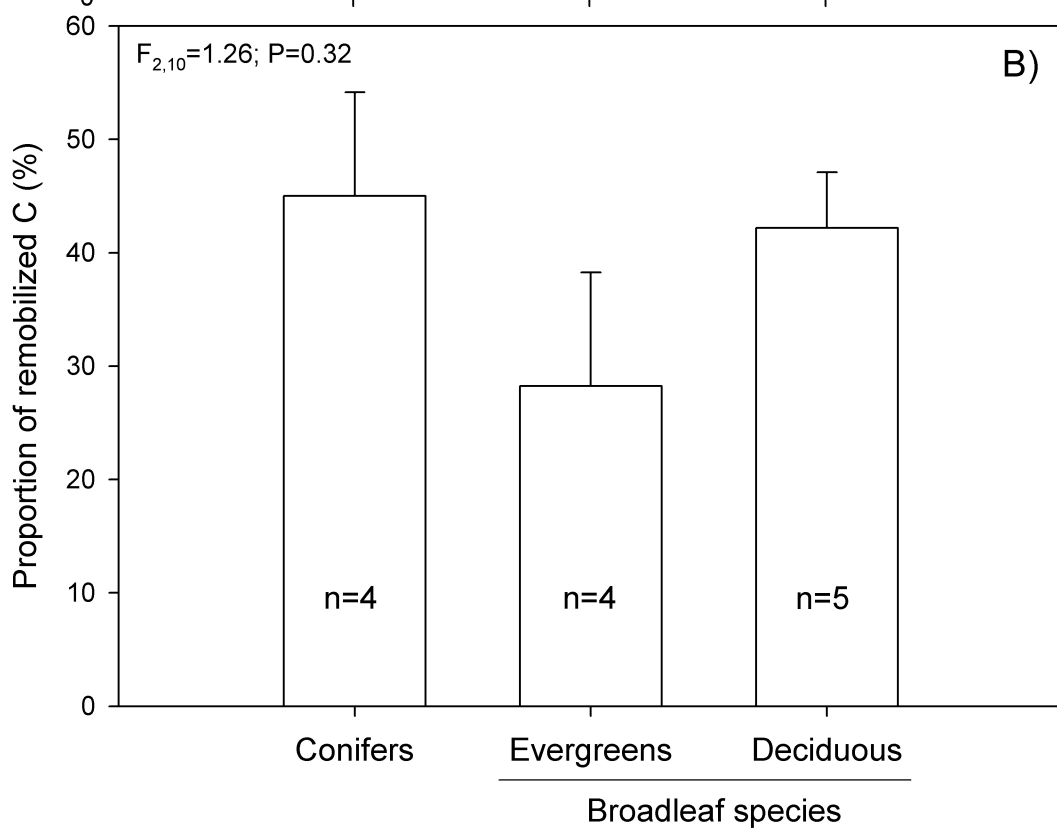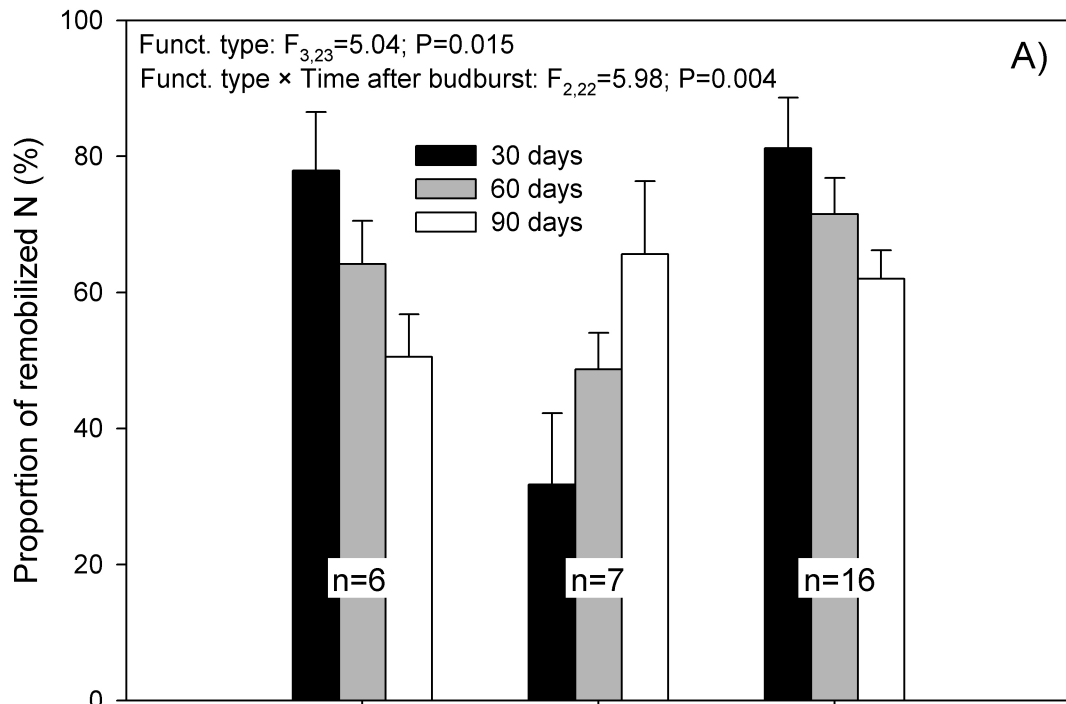|	Fall unfertilized seedlings	Fall N loaded seedlings	
At the end of cultivation			
Plant mass (g)	2.30 ± 0.18	1.91 ± 0.20	P=0.22
Shoot N concentration (mg g^{-1})	11.2 ± 0.29	26.8 ± 0.77	P<0.001
Plant N content (mg)	22 ±1.9	26.8 ± 0.77	P<0.001
Photosynthesis rate (μmol m^{-2} s^{-1})	2.31 ± 0.75	2.51 ± 0.74	P=0.84
Field performance in spring			
Photosynthesis rate (μmol m^{-2} s^{-1})	0.90 ± 0.14	0.28 ± 0.16	P=0.02
Uptake from soil N (mg)	3.9 ± 2.6	2.5 ± 2.5	P=0.67
N remobilization (mg)	3.8 ± 2.0	13.5 ± 1.6	P=0.007
Stem volume growth (ml)	0.17 ± 0.02	0.28 ± 0.02	P<0.001

10

1 Table 5. Summary of the most frequently effects (increase ↑, decrease ↓ or no effect =) of main
2 nursery practices on the concentration of nitrogen and NSC reserves. Effects with question marks
3 show hypothesized trends as no study on the effect of such nursery practice has been found.
4 Numbers indicate the references at the table footnote that support the specific effects. # In the
5 conventional and exponential fertilization regime, nutrients are supplied at a constant and
6 exponential rate, respectively through spring until the end of summer. In the fall fertilization regime
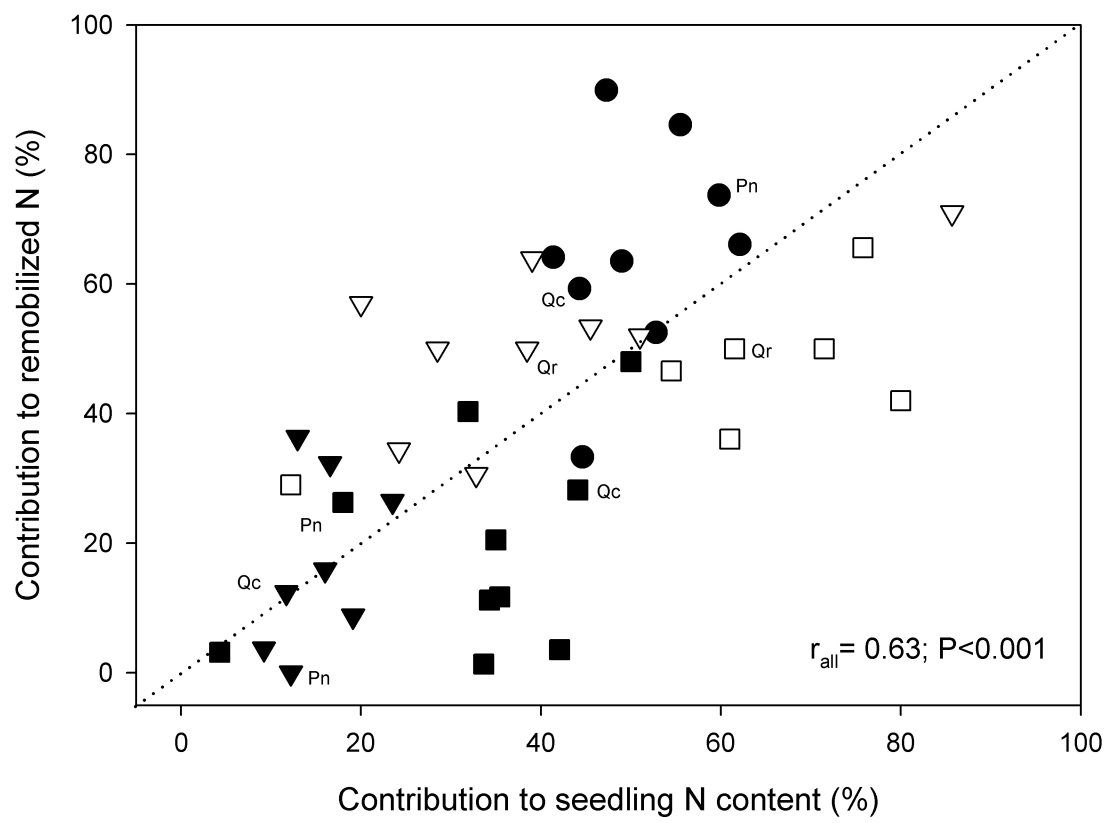7 nutrient supply continues through the fall.

Nursery practices	Effect on N reserves		Effect on NSC reserves		References
	Concentrat.	Content	Concentrat.	Content	
Fertilization					
Conventional/ exponential fertilization#	↑	↑	↓	= or ↓	(van den Driessche 1992; Sanz-Pérez et al 2007; Villar-Salvador et al 2013a; Oliet et al 2013; Heredia et al. 2014)
Fall fertilization	↑	↑	↑ ?	↑ ?	(Islam et al 2009; Oliet et al 2011; Li et al 2012; Andivia et al 2014)
Foliar fertilization	↑	↑	↓ ?	↓ ?	(Montville 1996; Uscola et al 2014; Wuyts et al 2015)
Hardening					
Irrigation: *moderate water stress for short periods*	↑	↑ or =	↑ or =	↑ or =	(Timmer and Miller 1991; van den Driessche 1992; Stewart and Lieffers 1993; Villar-Salvador et al 1999; Villar-Salvador et al. 2013b; Sanz-Pérez et al 2007; Sanz-Pérez et al 2009)
↓ Photoperiod	↑ or =	↑ or =	↑ or =	↓ or =	Grossnickle et al 1991; Tan 2007; Landhäusser et al 2012b).
↓ Light	↓	↓	↓	↓	Valladares et al 2000; Villar-Salvador et al 2004a; Baber et al 2014.
↓ Winter nursery temperature	↑	= ?	↑	= ?	Mollá et al 2006; Heredia et al 2014
Others					
Cold storage	= ?	= ?	↓	↓	Puttonen 1988; Cannell et al 1990;
↑ Container volume	↑ or =	↑	↑ or = ?	↑ ?	Robbins and Pharr 1988; Aphalo and Rikala 2003; Dominguez-Lerena et al 2006; Dumroese et al 2013
↓ Cultivation density	↑ or =	↑ or =	↑ or = ?	↑ ?	Dominguez-Lerena et al. 2006; van den Driessche 1984
Handling	↓	↓	↓	↓	Insley and Buckley 1985

8
9

Funct. type: $F_{3,23}=5.04$; $P=0.015$
Funct. type × Time after budburst: $F_{2,22}=5.98$; $P=0.004$

A)

- ■ 30 days
- ▨ 60 days
- ▢ 90 days

n=6

n=7

n=16

$F_{2,10}=1.26$; $P=0.32$

B)

n=4
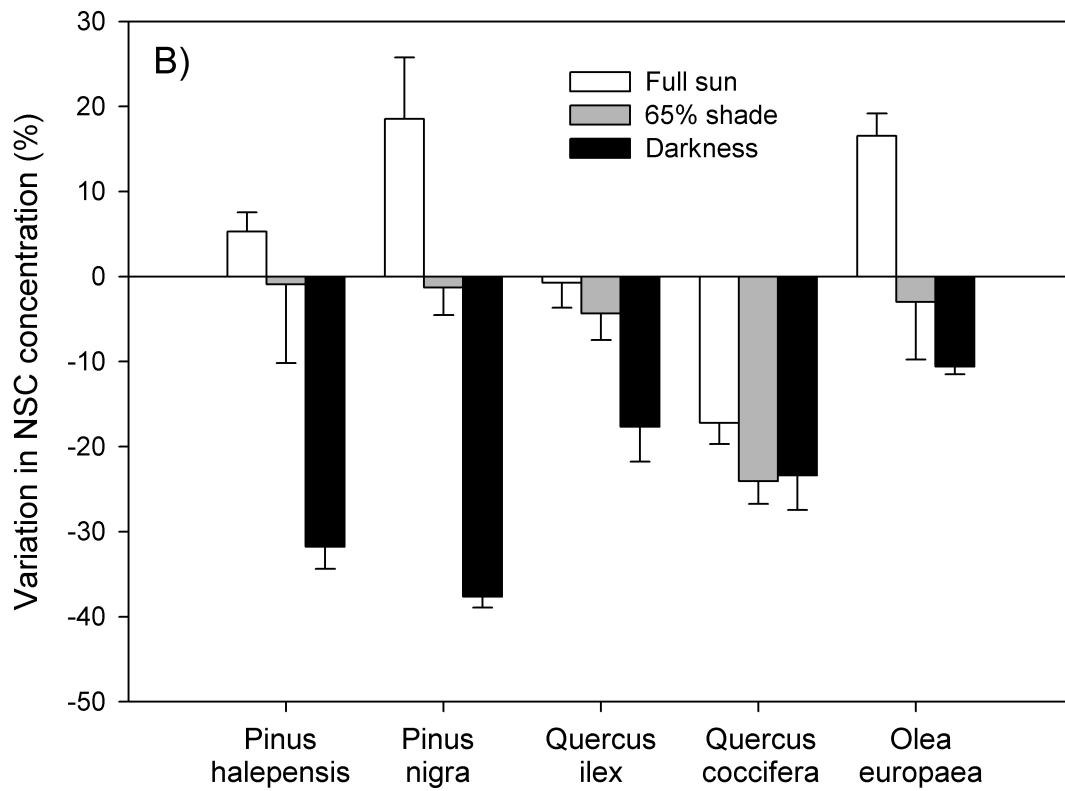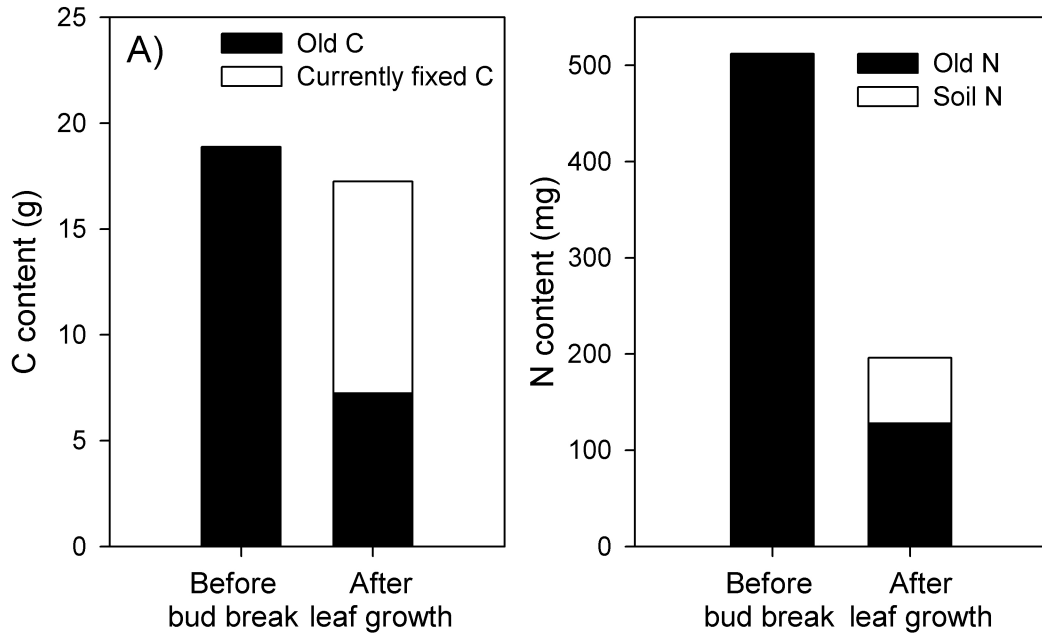
n=4

n=5

Conifers

Evergreens

Deciduous

Broadleaf species

A)

B)
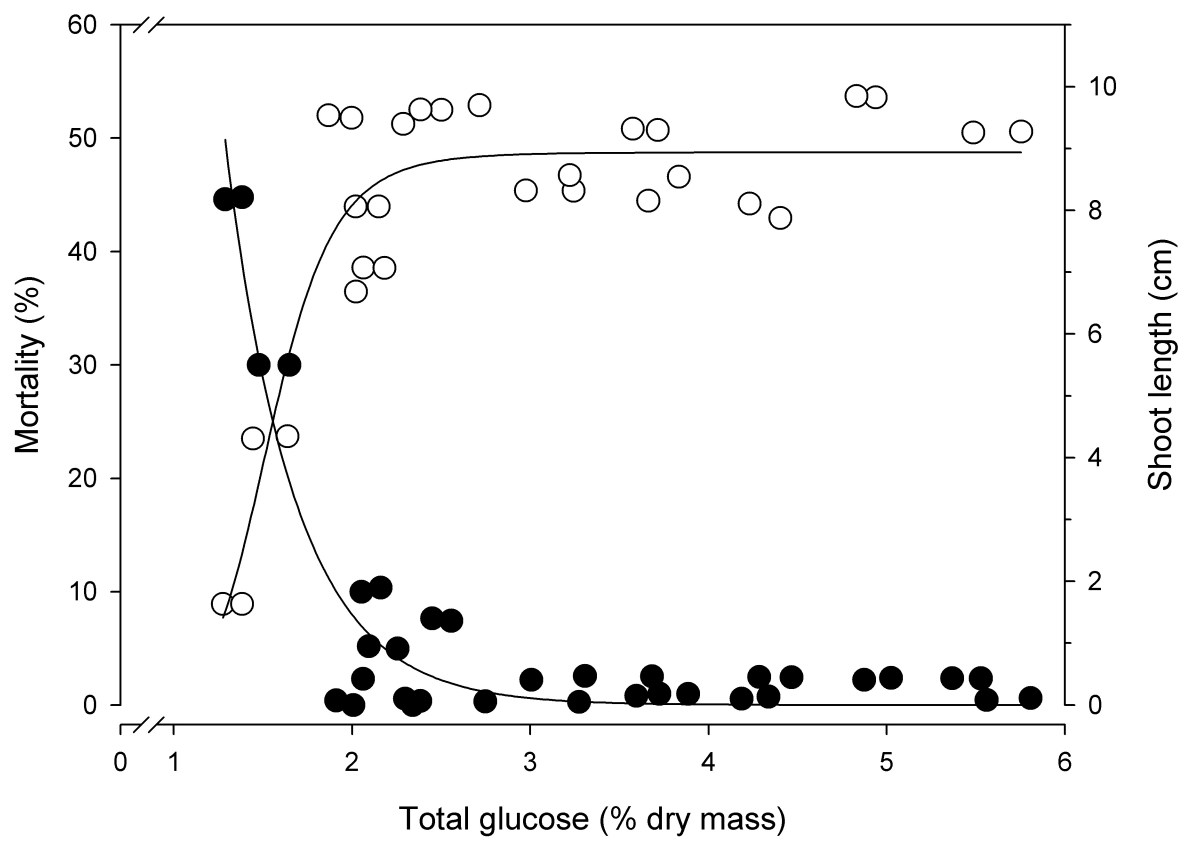
1 **SUPPLEMENTARY MATERIAL**

2 **Methodology for database creation**

3 **1) Partitioning of N and NSC into plant organs among main woody plant functional**

4 **types (Table 1)**

5 We extracted information from 41 studies encompassing 51 species for N and 42 species for

6 NSC data. We selected studies using 1 to 4-year-old plants with data on N and NSC

7 concentration and mass in at least leaves, stems and roots. We chose studies that had data

8 on plants sampled in the fall or winter, when seedlings were dormant or growth was arrested.

9 Many studies did not distinguish between leaves and stems and only provided data of

10 shoots. In these cases, we only used root data. Some information of Mediterranean species

11 (*Juniperus thurifera*, *Pinus pinaster*, *P. pinea*, *P. nigra*, *Pistacia lentiscus*, *Quercus faginea*,

12 and *Q. suber*) comes from unpublished data by P. Villar-Salvador. We calculated the N and

13 NSC content per plant compartment and finally calculated partitioning of such compounds on

14 a relative basis. In studies that used different fertilization regimes, we obtained the data from

15 moderate or high fertilization treatments. In studies that cultivated plants under increased

16 CO_2 we used the plants grown under ambient conditions. When the study had data of plants

17 of different ages we selected the data from 1 or 2-year old plants. If plants had been grown in

18 distinct locations (for instance in different nurseries) or had been cultivated under different

19 containers, we averaged treatment data.

20

21 **2) Contribution of stored N and C to the growth of new leaves/shoots and new roots**

22 **among main woody plants functional types (Figure 1 and Table 2)**

23 To study the contribution of stored N and C to the growth on new leaves/shoots and new

24 roots among main woody plants functional types, we extracted information from 27 studies

25 encompassing 32 woody species. Only long term labeling studies that used mixing isotope

26 models (Deléens et al. 1994; Fry 2006) were selected to assess the contribution of current

27 uptake and stored NSC and N on the growth of new fine roots and shoots. We did not

28 include studies that used a budget approach to quantify remobilization because this method

29 can only be used for N and it might provide significant bias in the calculation of the

30 contribution of remobilized N for new organ growth (see Millard 1996; Salifu & Timmer 2003).

31 Most studies used < 4-year-old plants (mainly 1 and 2-year-old plants) but in some cases we

32 used data of adult trees (Keel et al 2006) or the age of plants was unknown (Thornton et al

33 1995; Grelet et al 2001a; Palacio et al 2014). Some studies separated new leaves from other

34 new organs to analyze the contribution of remobilized resources to their growth, while others

35 analyzed the contribution of stored resources on the construction of new shoots. For

36 simplicity, we have joined new leaves and new shoots into the same category. In studies that

37 distinguished fertilization treatments and increased CO_2, data were obtained from high

fertilization and ambient CO_2 treatments, respectively. We also controlled for the sampling moment after bud break. Thus, in studies that examined the contribution of stored resources to new organ growth through time we used the data when the contribution of stored resources to new growth plateaued, which generally occurred at the end of the spring. Sampling time was a significant covariate for the contribution of stored N to new shoot/leaf growth. We used a separate slope model for the analysis of covariance as the measurement moment interacted with the functional type of woody plant.

3) Contribution of different organs to N remobilization (Figure 2 and Table 3)

The information was extracted from 10 studies encompassing 17 species. We used studies that had used N isotope labeling approach and quantified the content of old (remobilized) and new (soil N) N in the organs grown in previous growing seasons (leaves in evergreen taxa, and woody stems and roots) prior bud break and after complete leaf or shoot growth completion (at the end of spring or in summer).

4) References used for the construction of databases

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