Carry-over benefit of high internal N pool on growth and function of oak seedlings (Quercus petraea) competing with Deschampsia cespitosa

Vernay Antoinea, Malagoli Philippeb,*, Fernandez Marinea, Perot Thomasb, Amélio Thierrya, Balandier Philippeb

a Université Clermont Auvergne, INRA, PIAF, F-63000 Clermont-Ferrand, France
b Irstea, Research Unit on Forest Ecosystems (EFNO), Domaine des Barres, F-45290 Nogent-sur-Vernisson, France

ARTICLE INFO

Keywords:
Carry-over effect
Competition
Deschampsia cespitosa
Forest regeneration
Inter/intraspacific interactions
Internal N pool
Photosynthesis
Quercus petraea seedling
Water stress

ABSTRACT

In contrast to N soil availability or carbon (C) reserves, the role of N reserves in plant interactions has been under-researched. The N-loading of tree seedlings in nurseries before plantation within a forest community to increase their survival and early growth is still controversial. We hypothesized that a larger N-loaded pool in oak seedlings during year n – 1 might favor growth and function during year n through higher N remobilization, and thus lessen the impact of competition from Deschampsia cespitosa (tussock grass).

In 2015, we cultivated two sets of one-year oak seedlings in 201 pots with two nitrogen supply levels: no supply (N-unloaded) and 91 kg ha⁻¹ (N-loaded), without competition and without water limitation. A greenhouse experiment was then undertaken in 2016 with the same two oak batches, but with 0, 1, 2 or 3 tillers of D. cespitosa in both N-loaded and N-unloaded batches. Half the pots were subjected to drought for one month (August 2016).

Soil water availability was the prime factor driving plant growth and photosynthetic responses in oak, both with and without competition. Under well-watered conditions, a greater internal N pool was correlated with higher oak photosynthesis. A high internal N pool was also beneficial to oak seedlings under low competitive pressure from D. cespitosa. However, when grass density increased, intraspacific competition outweighed interspecific competition, and attenuated the negative effect of D. cespitosa on oak seedlings.

A carry-over effect of N-loading thus had a positive effect on growth and function in oak seedlings when no water stress occurred under low competition by D. cespitosa: this effect may derive from more N storage in perennial tissues, resulting in more N mobilized during vegetative growth. Our results underline the importance of the internal N pool in plant competition, and are evidence that a higher potential regenerative success rate of oak seedlings is related to abiotic conditions, competitive pressure and internal N pool.

1. Introduction

Competition between tree seedlings and grass in forest ecosystems has been well characterized and documented (Picon-Cochar et al., 2001; Coll et al., 2003; Balandier et al., 2006). Tree seedling survival and early growth can be massively jeopardized by strong competition from understory species (Provendier and Balandier, 2004; Balandier et al., 2006). Common grass response to clear-cut results in severe soil resource depletion through very fast growth and extensive root systems that rapidly prospect large soil areas and profiles (Freschet et al., 2017). Tree seedlings are much less efficient than grasses in taking up soil resources: they develop less rapidly and face low soil resource levels due to grass preemption. This leads to high seedling mortality rate or at least very poor early growth (Balandier et al., 2006; Harmer and Morgan, 2007; Frochot et al., 2009; Cuesta et al., 2010). To survive, tree seedlings thus resort to a lower allocation of carbon (C) and nitrogen (N) resources to growth and more resource storage in perennial organs (Shea et al., 2004): this allows C and N recycling to meet nutrient demand for growth later on, as environmental conditions improve.
Limited availabilities of light, soil water and nutrients, and their interactions impair seedling growth (Davis et al., 1998; Gaudio et al., 2011). For instance, because most minerals are available only from soil solution, dryness can directly affect N availability (Gonzalez-Dugo et al., 2005; Giehl and von Wieren, 2014; He and Dijkstra, 2014), especially in well-lit plots where competition between grass and tree seedlings for belowground resources is strongest (Vernay et al., 2016; Simon et al., 2017).

Competition from grass is likely to increase in the near future, because of (i) scarcer water resources, as drought frequency and intensity are expected to increase (IPCC, 2014), and (ii) the greater ability of grass to prospect and take up available N resources (Van Hees, 1997; Collet et al., 1999; Coll et al., 2003).

Stocking internal resource pools in tree seedlings, especially for N, has been proposed to counteract nutrient starvation due to rapid N depletion by competitive grass root uptake (Salifu and Timmer, 2001; Salifu et al., 2009; Ya-Lin et al., 2015): a rich internal N pool will thus act as a buffer compartment. N (and C) pools are well known to have a positive effect on plant functions under abiotic constraints, such as a better frost resistance (Morin et al., 2007; Mei et al., 2015), and an earlier and faster spring regrowth in roots (Millard and Proe, 1992; Malik and Timmer, 1998; Salifu and Timmer, 2001). Tree seedlings can also rely on this internal N pool to build new aerial organs during and after bud break (Staswick, 1994; Gomez and Faubert, 2002; Millard and Grelet, 2010; Bazot et al., 2016). N-loaded oaks (Quercus ilex L.) exhibited a higher N concentration at whole plant level, resulting in higher leaf area and photosynthesis performance (Guesta et al., 2010; Mei et al., 2015). Overall, an internal N pool may thus be a rescue option to cope with premature abiotic stress or nutrient starvation (Millard et al., 1990; Iqbal et al., 2012).

The beneficial effect of an internal N pool may have a residual effect: it has been shown that N-loaded trees such as Sitka spruce (Picea sitchensis) exhibit better aerial growth later in the season after bud break, two flushes being observed against only one for N-unloaded Sitka spruce (Millard and Proe, 1992). This could be interpreted as a carry-over effect of higher internal N pool recycling. Nevertheless, no study has yet evidenced any ecophysiological process related to the internal N pool that would explain this growth difference.

In grass, plants can also use N recycling to ensure regrowth in spring and keep a high level of growth and function irrespective of environmental nutrient availability. N reserves are stored in cord roots, internodes and seeds after aboveground biomass production ceases in summer. They are then recycled to new leaves after winter, as shown in Molinia caerulea in a fertile environment (van Heerwaarden et al., 2005). During the growing season, retranslocation of old leaf nutrients to new leaves enables grasses such as D. cespitosa (Davy, 1980) or Festuca rubra and Agrostis capillaris (Bausenwein et al., 2001) to offset nutrient soil deficiency.

To our knowledge, no study has yet explored the carry-over effect of the internal N pool on tree seedling growth and function during the subsequent vegetative season, in competition with grass. Some limited studies have focused on the functional role of the internal N pool in tree seedlings (Barbaroux et al., 2003; Millard and Grelet, 2010).

In this study we examined the role of the internal N pool of sessile oak seedlings (Quercus petraea) in competition with D. cespitosa. Two levels of internal N pools of oak seedlings were tested during the early interaction phase, a critical period for young trees, which drives their successful establishment and survival (Hanks et al., 2002). Two contrasting water regimes and three competitor densities were the other two factors of our experimental design. Both species are common in temperate forests, and are frequently found together in temporarily flooded stands (Davy, 1980). D. cespitosa often causes regeneration failure of Q. petraea, an oak species fairly tolerant to drought (Becker and Levy, 1982; Breda et al., 1993); D. cespitosa, though known as a hygrophilous species, is also reported to cope well with dry periods (Rahman, 1976; Davy, 1980). We focused on the early response of both species during six months of interaction.

We hypothesized that a well-stocked internal N pool in oak would improve seedling performance (growth and function) compared with a low internal N pool during early competition with D. cespitosa in both non-stressful and stressful contexts (here, drought and high grass density). The N pool accumulated during the previous year would be expected to produce higher leaf and root biomass and/or richer foliar tissues. More efficient first leaves would foster growth and resource capture early in the vegetative season, enhancing global plant performance under strong neighbor density and water stress.

2. Materials and methods

2.1. Experimental design and location

A greenhouse experiment with 20 L pots was set up at the INRA, UMR PIAF research unit in Clermont-Ferrand (Auvergne, France, 45°45'N, 3°07'E, altitude: 394 m). The experiment lasted from mid-May 2015 to September 2016 (Fig. 1). On 15 May 2015 we planted 144 one-year-old bare root oak seedlings (Quercus petraea Matt. (Leib.) (23 ± 0.5 g fresh weight per tree) sourced from a local tree nursery in 20 L pots (one seedling per pot) filled with a clay-sandy soil (clay 20.3%, loam 22.8%, sand 56.9%, pH 6.15, total N content 1.45 g kg\(^{-1}\), total C content 14.6 g kg\(^{-1}\)). They were cultivated in a sunny outdoor location in full light in 2015, and then transferred to a greenhouse in November 2015. Half the pots (72) received N fertilizer at an average rate of 29 kg ha\(^{-1}\) in May, June and July 2015 (i.e. 90 kg ha\(^{-1}\) in all, or 927 mg of inorganic N per pot) (N\(_{\text{EXT}}\)), while the other pots (72) received no N supply (N\(_{\text{INT}}\)) (Fig. 1). Our purpose was to favor the formation of N reserves in half the oak seedlings. To make sure this was

![Fig. 1. Time line of all measurements and treatment applications (\(\Phi_{\text{psi}}\) stands for predawn leaf water potential).](image-url)
achieved, we collected ten oaks in December 2015 and eight in March 2016. After drying and grinding, N content (%N) was measured in trees from harvested root systems (fine roots + taproot mixed as a single sample) and stems in N_{int} + and N_{int}, by a CHN elemental analyzer (Flash EA 1112 Series, THERMO-ELECTRON).

N amount per plant (N_{m}) was calculated by

\[ N_{m} = \%N \times DW_{\text{organ}}. \]  

Where \%N stands for N content expressed on a dry weight basis, and DW_{\text{organ}} the dry weight of the harvested organ.

N stock was defined as the difference in N_{m} in perennial organs between December 2015 (internal N pool assumed maximum due to storage of N retranslocated from senesced leaves and N taken up during autumn: N_{int}) and March 2016 (internal N pool assumed minimum due to massive retranslocation of N toward growing sink organs such as buds, leaves and roots: N_{int}) (Gerant et al., 2017). N_{int} was assessed after stage 95 of the international BBCH code (50% of leaves had changed color). N_{int} was assessed at budburst, stage 15 of the international BBCH code (50% of unfolded leaves) (Meier et al., 2009).

In December 2015, N_{int} was significantly higher in N_{int} + than in N_{int} −, as whole plant main (partially due to root stock difference, data not shown). In March 2016, N_{int} was similar in N_{int} + and N_{int} −, oak seedlings (Fig. A1). Hence, N stock in N_{int} + was higher than N stock in N_{int} − (0.45g > 0.22g). N stock could be remobilized in new organs or used for maintenance during dormancy. However, since the two oak groups (N_{int} + and N_{int} −) had the same abiotic environment and showed no growth difference, we considered that they used the same amount for metabolic maintenance, and the N amount remobilized at budburst was higher in N_{int} + than in N_{int} −.

2.2. Competition design

*Deschampsia cespitosa* (L.) tufts (aboveground parts + roots) were carefully collected under natural forest conditions at Paray-le-Fresil (Auvergne, France; 46° 39’ N, 3° 36’ E), and transplanted into the oak pots on 7 March 2016. Oak seedlings were grown either without *D. cespitosa* (L.) or with different grass densities in terms of tiller numbers, treatment (T), 1.1 ± 0.04g per tiller fresh weight of *D. cespitosa*: 0 T (n = 36 seedlings), 1 T (n = 18), 2 T (n = 18), and 3 T (n = 18).

2.3. Water stress treatment (W)

Until August 2016, water was provided close to the field capacity to prevent water stress. Irrespective of *Deschampsia cespitosa* density, half the pots then underwent water stress (W −) to mimic a drought period for one month (August 2016). Soil water content (SWC) at field capacity and wilting point in pots were measured with reflectometry probes (Spectrum WaterScout SM100): 38% and 12% of soil water content (SWC), respectively. We used these values to calculate the relative extractable water content REF (Vicca et al., 2012), an index that takes into account soil ability to retain water to assess extractability of water; 0.4 is considered as the threshold value below which water is assumed to be limiting in the soil (Granier et al., 2007; Vicca et al., 2012). We restricted water supply to reach REF values below 0.4 (i.e. < 22.9% SWC) in the W − treatment. During drought, SWC ranged from 29.5% (REF = 0.61) when the watering system was stopped (1 August 2016) to 13.7% (REF = 0.03) (daily mean) at the end of the drought period (1 September 2016, W −) (mean during drought 17.1% ± 0.8%, REF = 0.16) (Fig. A2). The other half of the pots were well-irrigated (W + treatment), with REF > 0.4. SWC reached 35.7% (REF = 0.84) (1 August 2016) and 28.4% (REF = 0.57) (1 September 2016) (daily mean) during the same period (mean during drought 32.7% ± 0.3%, REF = 0.73) (Fig. A2).

2.4. Predawn leaf water potential measurements

During the drought period, predawn leaf water potential (Ψ_{pd}) was measured weekly in all the treatment combinations. Two leaves per species were collected before sunset, and Ψ_{pd} was estimated with a pressure chamber (Scholander et al., 1965). The mean value of the two measured leaves per plant was used as single replicate.

In W −, Ψ_{pd} values were similar over the 5 weeks (mean value −0.5 ± 0.02 (SE) MPa). No difference was observed between the two species, except at the second week (−0.6 ± 0.03 (SE) MPa vs −0.3 ± 0.03 (SE) MPa for oaks and *D. cespitosa*, respectively) (Fig. A3). This difference could be explained by an increase in temperature before watering system adaptation.

In W −, water shortage resulted in a decrease in Ψ_{pd} from the second week for oaks and from the third week for *D. cespitosa* (Fig. A3); Ψ_{pd} values ranged from −0.3 ± 0.04 (SE) MPa to −1.8 ± 0.06 (SE) MPa for *D. cespitosa* and from −0.4 ± 0.03 (SE) MPa to −2.6 ± 0.06 (SE) MPa for oak seedlings. We noted no significant difference between species. As soon as water was re-supplied (at the end of the fourth week), Ψ_{pd} values were less negative, reaching a value similar to W + in *D. cespitosa*, and an intermediate value in oak leaves, close to Ψ_{pd} observed after only one week of drought.

2.5. N Fertilization

N fertilizer was supplied because of N scarcity at the end of 2015 (9 ± 1 mg kg⁻¹ of NO₃⁻ in soil and 4 ± 0.2 mg kg⁻¹ of NH₄⁺) in the pots: oak seedlings had been taking up resources for one year and soil inorganic nitrogen had been leached from the pots during the outdoor experiment. N fertilizer was evenly distributed over the pot surface of all the pots twice in 2016, with a solution of NH₄NO₃. In all, 85kgNha⁻¹ was provided (818mg of inorganic N per pot or 0.004kgm⁻²); the first application corresponded to 58kgNha⁻¹ (July 6, 2016), the second to 26kgNha⁻¹ (July 29, 2016, Fig. 1).

2.6. ¹⁵N supply and N content

Isotopic labeling was used to assess how N taken up during the drought period was distributed in the foliage of each species. ¹⁵NO₃⁻/¹⁵NH₄⁺ (42mg of ¹⁵N dissolved in 500ml of water) was evenly supplied at the surface in 70 pots on 29 July 2016. Total N content and ¹⁵N isotopic abundance were determined only on the foliar compartment, with an IRMS on PTEF OC 081 (INRA Nancy-Lorraine, EEF team, Champenoux). Leaf samples collected at the beginning of the first week (02/08/2016), the second week (09/08/2016) and the fifth week (last week, 30/08/2016) of the drought period were analyzed. Labeling methods and associated calculations are detailed in Vernay et al. (2016).

2.7. Inorganic soil nitrogen

We estimated inorganic soil nitrogen (N_{int}) at the beginning (2 August) and at the end of the drought period (29 August). We sampled 20cm soil cores in the middle of the pot in each N_{int} × T × W (n = 5 per treatment combination). Samples were frozen and stored at −20°C and then analyzed with the Berthelot color reaction (Weatherburn, 1967) for ammonium, and ionic chromatography on METROMOM chain analysis (Metrohm AG Oberdorfstrasse 68 CH-9101 Herisau) for nitrates. At the beginning of the drought treatment, NO₃⁻ (n = 85) and
NH₄⁺ (n = 85) soil content were respectively 6 ± 1 mg kg⁻¹ of dry soil and 3 ± 0.3 mg kg⁻¹. At the end of the drought treatment they were 11 ± 2 mg kg⁻¹ and 6 ± 0.2 mg kg⁻¹ respectively, with no significant effect of any treatment (data not shown).

2.8. Measurement of leaf maximum carbon assimilation (Amax) and stomatal conductance (gₛ)

Leaf gas exchange measurements were performed weekly over all treatments (n = 3 per W×T×N₅₉ combination), during the drought period. Maximum assimilation (Amax) and stomatal conductance (gₛ) were measured with a Li-Cor 6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA). Photosynthetic active radiation (PAR) was set at 1500 μmol m⁻² s⁻¹ to mimic high light conditions, and CO₂ supply was maintained at 400 ± 0.05 ppm. Vapor pressure deficit (VPD) was 1.1 ± 0.01 kPa, and leaf temperature 24.6 ± 0.1 °C. Two leaves per species per pot were measured, and the average of these two measurements was used as a replicate in analysis (i.e. one value per species per pot). For D. cespitosa measurements, several leaves were gathered to fill the measurement window with minimal gaps or overlapping to obtain values comparable to a single oak leaf surface.

2.9. Growth measurement

Length of stem and collar diameter of oak seedlings and length as the highest leaf and tuft diameter for D. cespitosa were measured every 10 days during the experiment. We used measuring tape for length measurements and callipers for diameter measurements at fixed stem level. Relative growth rate (RGR) was calculated as:

\[
RGR = \frac{\ln(x_t) - \ln(x_i)}{t_2 - t_1},
\]

with x the plant length or diameter, t₂ the end of drought date, and t₁ the date of plantation.

2.10. Plant harvesting

In mid-September 2016 (Fig. 1), leaves were counted and collected from all oak seedlings. All the aboveground biomass of D. cespitosa was harvested. Plant material was dried at 60 °C for at least 48 h and then weighed for both species.

2.11. Statistics

A crossed factorial design with three factors was used: water (W = W⁻/W⁺), internal N pool (N₅₉⁻/N₅₉⁺), and D. cespitosa density (0/1/2/3 tillers per pot noted 0T/1T/2T/3T, respectively). Preliminary tests demonstrated no effect of spatial position of pots in the greenhouse in terms of air temperature, light availability or relative humidity. D. cespitosa density (T), water stress (W) and preliminary fertilization (N₅₉) were implemented as fixed effects in a linear model. ANOVA type III and multiple mean comparison estimated single factor effects and their interactions. Pots were stood six to a pallet (subplots) for practical convenience, and so subplot was integrated in our statistical model as a random effect. Our statistical model considered all factors and factor interactions. Insignificant higher-order interactions were removed to simplify the model according to each studied variable. Simplified models were then fitted with the restricted maximum likelihood method (REML) to better estimate variance components (Pinheiro & Bates, 2000). The lime function of the lme package was used to fit the linear mixed effect models (Pinheiro et al., 2016).

Because the establishment of the water stress was gradual, we used data from the end of the period (when stress was highest) in the linear mixed model. Data were analyzed using R statistical software (R Core Team, 2015).

3. Results

3.1. Response of plant growth and function to water availability, internal N pool of oak seedlings and tiller density (Table A.1 and A.2)

In W⁻, Amax and gₛ were lower for both species than in W⁺ (Table A.1a and A.2a). In oak, leaf dry weight was also lower in W⁻ than in W⁺, whereas in D. cespitosa values of length RGR and diameter RGR were significantly lower in W⁻ (Table A.1a and A.2a), with no change in leaf dry weight.

In N₅₉⁺, leaf dry weight and diameter RGR, together with Amax and gₛ, were higher for oak and lower for D. cespitosa than in N₅₉⁻ (Table A.1a and A.2a).

D. cespitosa density had a negative effect on leaf dry weight in oak beyond 2T. It also led to lower oak leaf RGR in 3T compared with 0T; gₛ in oak was significantly lower only for density 1T (Table A.1a).

We noted that when tiller density increased to 2T, total D. cespitosa leaf biomass was also higher. However, no statistical difference appeared between 2T and 3T; a third tiller did not increase total leaf biomass of grass (Table A.2a).

3.2. Response of Amax in oak and D. cespitosa to W×N₅₉ (Fig. 2) and T×N₅₉ interactions (Figs. 3 and 4)

In oak no effect of W×T combination was observed (Table A.1c) on Amax. By contrast, Amax values were much more responsive to W×N₅₉ (Fig. 2, (Table A.1b) and T×N₅₉ (Figs. 3 and 4, Table A.1d): Amax was higher in W⁺/N₅₉⁺, and for low grass density with N₅₉⁻.

For N₅₉×T interaction treatment, results show that oak Amax did not respond to number of tillers in N₅₉⁻, whereas Amax values were higher when oak was alone (i.e. 0T) in N₅₉⁻; Amax was then lowest for 2T (Fig. 3, Table A.1d), close to N₅₉⁻ values.

In D. cespitosa, in the N₅₉⁻ treatment, maximum values for Amax were measured for 1T; they then reached a minimal value for 3T (Fig. 4, Table A.2d). By contrast, in N₅₉⁺, treatment, Amax values were drastically lowered for 1T compared with N₅₉⁻. This value did not significantly change according to density (Fig. 4, Table A.2d). At the high-

---

Fig. 2. Water treatment (W) effect in interaction with internal N pool (N₅₉) on oak seedling maximum assimilation (Amax). White bars are N₅₉⁻ (N-unloaded) oak seedlings and black bars N₅₉⁺ (N-loaded) oak seedlings. Values are reported as means ± se (n = 43). Different letters correspond to a statistically significant difference between W×N₅₉ with p < 0.05.
est density, $A_{\text{max}}$ values were in the same range for either N$_{\text{INT}}$ treatment.

Overall, $A_{\text{max}}$ values were higher for D. cespitosa than for oak seedlings ($p < 0.0001$, data not shown).

### 3.3. Response of $g_{s}$ in oak to $W \times N_{\text{INT}}$ (Fig. 5) and D. cespitosa to $W \times T$ (Fig. 6)

In oak, in the N$_{\text{INT}}$ treatment, regular supply of water had no effect on $g_{s}$ versus the drought treatment (Fig. 5, Table A.1b). By contrast, in the N$_{\text{INT}}$ treatment, supply of water close to field capacity led to a higher $g_{s}$ almost 2.7-fold more than in the other treatment combinations. Under W$-$ treatment, higher internal N pool of oak had no effect on $g_{s}$ (Fig. 5, Table A.1b). Again, like for $A_{\text{max}}$ values in oak, a positive effect on $g_{s}$ in oak was observed when soil water was fully available.

D. cespitosa density did not affect grass $g_{s}$ in either W$+$ or W$-$; the only significant difference was observed between 1T/W$-$ and 3T/W$+$, where $g_{s}$ was higher in 3T/W$+$ (Fig. 6, Table A.2c).

### 3.4. $^{15}$N labeling (Fig. 7)

$^{15}$N was supplied at the beginning of the water shortage (Week 1, 1 August 2016) for both species. In both species, $^{15}$N was measured in leaves in Week 1, four days after supply; $\delta^{15}$N values were in the same range for both species, 656±152‰ vs. 1704±238‰ for oak and D. cespitosa, respectively. However, during Weeks 2 and 5, leaf $\delta^{15}$N values were much higher in D. cespitosa than in oak ($p < 0.0001$); $\delta^{15}$N values were not statistically different between Weeks 2 and 5 in either species (Fig. 7).
4. Discussion

4.1. Carry-over effect: how can N absorbed in the previous year benefit current growth?

Mobilized N from storage tissues during regrowth after bud break was greater in trees receiving N supply the year before than in trees receiving no N supply. Interestingly, most recycled N was allocated to leaves, in line with other studies (Berger and Glatzel, 2001; Salifu and Timmer, 2001; Grunzweig et al., 2008; Vizoso et al., 2008). In March 2016, no budburst offset in terms of date was recorded between $N_{\text{NT}^+}$ and $N_{\text{NT}}$ groups (data not shown). They also underwent the same dormancy conditions and reached the total biomass in March. We suggest that the $N_{\text{NT}^+}$ group used its higher N stock to build new leaves at the beginning of the vegetative season: it is well established that growth of new organs (especially leaves) in deciduous woody species greatly relies on internal N-resource reallocation before reaching the autotrophic stage during and after bud break, depending on species (Jordan, 2015; Bazot et al., 2016). Such improvement in N can result not only in an early improved specific leaf area such as in poplar (Thitithanakul et al., 2012) but also in a greater foliar N content: this may then trigger more efficient intrinsic leaf photosynthesis processes and subsequent carbon capture from budbreak. In conclusion, it can be suggested that leaf C capture might be enhanced due to rapid, larger N provision to leaflets fueled by greater N mobilization from internal N recycling in oak seedlings with higher N reserves. This might allow more efficient and more promptly switched-on leaf photosynthesis-related processes.

Oak seedlings with a higher internal N pool exhibited a higher stem diameter RGR than those with low N stock, consistent with other studies (Cuesta et al., 2010); higher internal N pool remobilization would develop more photosynthetically efficient leaves (see previous section), with a direct effect on stem diameter. Moreover, several studies have demonstrated a relevant allometric relation between stem diameter and root system biomass (Drexhage et al., 1999; Enquist and Niklas, 2002). Fine root growth could have been fostered by N allocation earlier in the vegetative season (compared with $N_{\text{NT}^-}$) and by earlier C gain coming from new leaves (Cuesta et al., 2010; Oliet et al., 2013). Earlier root growth, triggered by early photosynthesis, leads to earlier uptake of nutrient and water in the soil before grass depletes it. A virtuous circle involving growth and function (in above- and belowground compartments) would then confer greater efficiency in meeting grass competition.

These responses could be ascribed to a carry-over effect of the internal N pool stored the previous year, conferring better leaf quality and so prompter functioning, with a richer resource pool.

4.2. High internal N pool, an advantage for oak seedlings in well-watered conditions

As reported in some studies, a high level of N remobilization offers an advantage for tree seedlings in the event of abiotic stress or competition (Cuesta et al., 2010; Oliet et al., 2013). Nevertheless, a high internal N pool was beneficial to leaf parameters ($A_{\text{max}}$ and $g_{\text{c}}$) only in well-watered conditions, which is partially consistent with our hypothesis. Our results thus showed that $g_{\text{c}}$ was higher only in well-watered pots, concomitantly with the $A_{\text{max}}$ value. Limitation of the positive effect of the internal N pool in oak seedlings by soil water availability and/or number of $D. cespitosa$ tillers on $A_{\text{max}}$ and $g_{\text{c}}$ raises questions about the processes involved. Several hypotheses can be advanced. First, under limited soil water availability, one common response is to enhance root growth to explore a larger soil volume, and ultimately to take up more water (Ho et al., 2005; Hodge, 2010). Such growth will require larger C and N supplies toward roots to the detriment of leaf C and N supply. Secondly, water potential reached values as low as $-2.6 \text{MPa}$, implying a severe water stress in oak despite its relative tolerance to drought compared with other species of the same genus (Dickson and Tomlinson, 1996; Picon et al., 1996). It might thus be that mobilization of N compounds from reserve tissues (such as coarse root and bark) are slowed and/or delayed due to low levels of hydration of these tissues, thus jeopardizing cell metabolism including proteolysis and amino acid conversion to transport. Specific biochemical experiments would be needed to test this hypothesis.

Thirdly, a common response is that water stress induces lower assimilation and conductance because of stomatal closure to save water and avoid cavitation (Dreyer et al., 1992; Timbal and Aussenac, 1996; Siam et al., 2009). By contrast, under well-watered conditions, photosynthetic performance was significantly improved for larger internal N pools in oak seedlings. Well-watered oaks avoided stomatal closure, enhancing gas exchange (Cholard et al., 1996; Klein, 2015). The carry-over effect of a high internal N pool in leaves was then optimized, well-performing photosynthetic metabolism and hydraulic supply acting together.
4.3. Relationship between the impact of interspecific and intraspecific competition on growth and development

Testing different D. cespitosa tiller densities, we hypothesized stronger competition for soil resources between oak seedlings and grass with increasing tiller number, as postulated in other studies (Davis et al., 1998; Balandier et al., 2006; Lof et al., 2014). Concerning oak, leaf biomass was clearly lower when oak was grown with 1T and 2T. Maximal threshold stress affecting foliar biomass may have been reached from 2T. Three tillers in the pots did not affect oak leaf biomass more negatively than 2T treatment. This would be consistent with a too-high grass density, increasing grass interspecific competition and so down-regulating ecophysiological processes and resource access. Competitive pressure would already be maximal with 2T on oak and would be greater on grasses with a supplementary tiller than on oak. This could explain the trend to higher \( A_{\text{max}} \) for \( N_{\text{INT}} \), oak with 3T. Concerning D. cespitosa, leaf biomass did not increase between 2T and 3T despite an additional tiller. Accordingly, a hypothesis of stronger intraspecific competition may be put forward for the highest D. cespitosa density. This is consistent with interspecific competition between D. cespitosa tillers outweighing interspecific competition between D. cespitosa and oak, and a stabilizing process of co-occurrence (Maire et al., 2012), although some scientists disagree (Weigel et al., 2007)). Biotic interactions lead to auto-regulation of competitive pressure among species, inducing lower resource demand of the grass (too-high density) and hence a lower growth. D. cespitosa was much more competitive than oak seedlings, but beyond a given density threshold under our experiment conditions, resources failed to feed all tillers, leading to negative effects on D. cespitosa. A lower resource depletion by grasses would reduce harmful effects of soil resource starvation (less N uptake by grass) on oak seedlings, which could in turn enjoy a higher resource availability. Because interspecific and intraspecific competition occur concomitantly in the community, we assume that interspecific competition was stronger than intraspecific at low density levels. This pattern then reversed: net outcomes of intraspecific competition outweighed those of interspecific competition at high population density (HilleRisLambers et al., 2012).

The response of grass to density can be explained by different mechanisms other than resource depletion. Chemical or organic signals in the rhizosphere can be interpreted by plants to detect the presence of neighbors, and induce positive or negative growth response (Delory et al., 2016). For instance, D. cespitosa growth is reduced in the presence of siblings, whereas high growth rate is maintained with allospecific neighbors (Semchenko et al., 2014): a trade-off between all chemical signals might influence grass performance.

4.4. Inter- and intraspecific competition: impact on plant function

At low D. cespitosa density, better performance of oak seedlings with a high internal N stock lent them a higher competitive ability. As shown by Vernay et al. (2016), \( A_{\text{max}} \) and quantum yield are strongly correlated to N supply in leaves. It can then be hypothesized that a higher internal N stock may improve leaf Rubisco content in oak, resulting in an improved \( A_{\text{max}} \) value. Subsequent C gain may result in an improved growth of oak seedlings, including RGR in diameter positively correlated to root growth (Drexhage et al., 1999; Enquist and Niklas, 2002) leading to a virtuous loop between N uptake and photosynthetic performance. However, as the density of D. cespitosa increased, competitive abilities of grass decrease N resource access to oak seedlings, reducing their photosynthetic abilities, at least until 2T. Finally, for the highest D. cespitosa density (3T), oak showed functional performance close to that measured at low grass density, not because of higher competitive abilities, but because of higher intraspecific competition between D. cespitosa tillers. Similar \( A_{\text{max}} \) values of D. cespitosa when they grew with \( N_{\text{INT}} \), oak (a positive trend was observed between \( A_{\text{max}} \) value and tiller number associated with significant \( T \times N_{\text{INT}} \) interaction) would support the hypothesis of a potential positive effect of oak seedlings on D. cespitosa. A higher root turnover and/or higher rhizodeposition (Gross et al., 1993; Priyadarshini et al., 2014; Karst et al., 2016) would provide an extra N source for grass.

Under field conditions, grass cover as D. cespitosa is often quite dense (close to 2T and 3T, in terms of biomass), thus lessening the sharply positive effect of a high internal N pool in oak. A trade-off might thus be to replant oak seedlings with a high internal N pool associated with basic mechanical management to keep D. cespitosa under a set threshold, thus reducing competitive pressure on oak seedlings.

5. Conclusion and perspectives

This study set out to assess the role of a high internal N pool accumulated the previous year on oak seedling growth and biomass in competition with D. cespitosa during the following vegetative season. A larger internal N pool was correlated with higher photosynthetic performance. This resulted in better oak seedling growth through more efficient leaf and root systems from budburst time. This positive carry-over effect was expressed only in well-watered pots and for relatively low density of neighbors. Overall, water was the foremost driver of plant function in this study. Interactions between plants switched from higher interspecific to higher intraspecific competition with increased D. cespitosa density. In silvicultural management practice, plantation of high internal N pool oak seedlings would offer an effective solution to resist competition by perennial grasses only at low grass density, and with no water stress. A focus on both growth and function in root systems, from budburst to senescence, would help clarify whether the N pool has an effective role in dealing with stress in the belowground compartment, and from what stress intensity the internal N pool is solicited (Shea et al., 2004). The possible positive role of N-loaded oak on D. cespitosa remains unclear and deserves more research, in particular a potential role of N transfer from tree seedlings to understory species and organic N sources.

Authors’ contributions

AV, PM, TA and PB originated the research and designed the methodology; AV, PB and MF collected the data; AV, TP, PM, TA and PB analyzed the data; AV, PM, TA and PB wrote the manuscript. All the authors contributed critically to the drafts and gave final approval for publication.

Declarations of interest

None.

6. Uncited reference

Jensen et al. (2011).

Acknowledgments

The authors thank André Marquier, Christophe Serre, Aline Faure, Patrice Chalée, Brigitte Saint-Joanis, Anais Hubert, Marc Vandame and Pascal Walser for help in preparing the greenhouse set-up, daily managing of pot watering, weekly measurements of plant growth, plant and soil harvesting, data collection and sample processing, and Jérôme Ngao for help and advice during Licor measurements. The authors thank the European Agricultural Fund for Rural Development (FEADER Leader), the French Ministry of Agriculture, the Regional Direction of
Agriculture of Auvergne (DRAAF), and the Allier department (CG 03) for financial support. They are grateful to Philippe Charrier at Le Moulin de Grandveau (Paray-le-Fresil, France) for allowing us to harvest D. cespitosa from private woodland. The authors thank the certified facility in Functional Ecology (PTF EF 081) of UMR 1137 EEF and UR 1138 BEF at the INRA Nancy-Lorraine research centre for its contribution to isotopic analysis. Antoine Vernay was supported by a French Ministry Research grant (Doctoral school SVSAE). Lastly, the authors thank the anonymous reviewers for their advice and comments.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.foreco.2018.03.039.

References


