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The effects of lifting on mobilisation and new assimilation of C and N during regrowth of transplanted Corsican pine seedlings. A dual ^{13}C and ^{15}N labelling approach

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Abstract – A dual long-term ^{13}C and ^{15}N labelling procedure was used to label C and N reserves of one-year-old Corsican pine seedlings. After labelling, seedlings were either submitted to a removal of root tips to simulate a nursery lifting or kept intact before being transplanted into minirhizotrons (parallelepipedal containers allowing root elongation observations) and grown for 41 days in a climatized chamber. Applying isotopic dilution equations allowed to assess for regrowth the effects of lifting on the use of C and N either derived from reserves or newly assimilated. Lifting decreased markedly root and shoot growth after transplanting, as well as CO_2 assimilation rate and stomatal conductance of needles. However, both the ratio of needle intercellular/ambient CO_2 concentration and needle predawn water potential remained unaltered. Lifting also decreased total non-structural carbohydrate (TNC) concentration at the whole seedling level. Shoot growth began just after transplanting and was supported by old C. Concomitantly, a strong decrease in old C and TNC occurred in old roots. A marked N mobilisation was also observed in needles. New root growth occurred 15 days after transplanting and depended less on old C and N than did the new shoot growth. Shoot and root growth were further supported more and more by newly acquired C and N. On day 41 after transplanting, new roots contained mainly new C and N, while new shoots remained enriched in old N. The decrease in growth in response to lifting was more pronounced in roots than in shoots and resulted less from a reduced availability of reserves than from a significant decrease in acquisition of both C and N.

transplanting stress / growth / C / N / assimilation / remobilisation

Résumé – Impact d'un arrachage sur le déstockage et l'assimilation de C et d'N lors de la reprise de croissance après transplantation de plants de pin corse. Utilisation d'une méthode de double marquage ^{13}C ^{15}N . Un double marquage à long terme à l'aide d'isotopes stables (^{13}C , ^{15}N) a été appliqué à des plants de pin laricio de Corse âgés d'un an afin de marquer leurs réserves carbonées et azotées. Les extrémités racinaires blanches des plants ont ensuite été soit coupées, simulant un arrachage des racines en pépinière, soit maintenues intactes. Les plants ont alors été transplantés dans des minirhizotrons (conteneurs parallélépipédiques permettant l'observation de l'allongement des racines) et installés pendant 41 jours dans une chambre climatisée. La dilution isotopique liée à l'entrée de C et de N non marqués, a été suivie après transplantation afin de déterminer l'impact de l'arrachage sur la nouvelle croissance et la gestion du C et de l'N issus des réserves ou de l'assimilation. L'arrachage des racines a abouti à une diminution des croissances aérienne et racinaire. L'assimilation nette de CO_2 et la conductance stomatique ont été diminuées dans les aiguilles. Cependant, ni le rapport des concentrations intercellulaire foliaire/atmosphérique en CO_2 ni le potentiel hydrique de base des aiguilles n'ont été affectés par l'arrachage. La concentration en glucides non structuraux des plants entiers a diminué. L'allongement de la partie aérienne a débuté juste après transplantation avec utilisation principale de C ancien. Concomitamment, les quantités de C ancien et de glucides ont diminué fortement dans les racines préexistantes. Une mobilisation marquée de l'N ancien des aiguilles a également été observée. La croissance des nouvelles racines a débuté 15 jours après transplantation avec une utilisation moindre de C et N anciens comparativement à la nouvelle pousse aérienne. Ensuite, les nouveaux organes ont incorporé essentiellement du C et N nouveaux. Après 41 jours de croissance, les racines contenaient surtout du C et N nouveaux alors que la nouvelle pousse restait riche en N ancien. La diminution de croissance induite par la transplantation a concerné plus les nouvelles racines que la nouvelle pousse, et a résulté moins d'une diminution des réserves disponibles que d'une diminution de l'acquisition et de l'utilisation de C et d'N nouveaux.

stress de transplantation / croissance / C / N / assimilation / remobilisation

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1. INTRODUCTION

Afforestation or reforestation often requires the use of nursery-produced plants. Bare-root tree seedlings previously grown in nursery beds, and then lifted, are preferentially used by foresters due to their low cost compared to those of container-grown seedlings [3]. In these seedlings, root systems generally remain without the protection of soil during a variable time until their final planting in forest sites.

Practices associated with mechanical handling disrupt the functional continuity between soil and roots, and cause root injuries with loss of fine roots, resulting in reduced water and nutrient absorption [21]. Lifting and transplanting of bare-root stock, with or without storage, can compromise subsequent survival and growth in the field [1, 27] through a series of physiological and metabolic disorders [20]: Ecophysiological studies point to the importance of a rapid growth of shoots and roots to recover efficient water, carbon, and nutrient acquisition [9, 20, 21, 31]. These studies have stressed the importance of new root growth for the establishment of transplanted tree seedlings [2].

After transplanting, lifted seedlings will require carbon and nitrogen compounds to support new root growth and associated energy needs. In contrast with seedlings of deciduous tree species, coniferous seedlings are able to photosynthesise in mild late winter and early spring conditions [17, 19]. Consequently, coniferous seedlings may use both currently produced or stored C sources. The importance of these two sources for new growth can vary by species [24], but several studies have reported that transplanted seedlings are often water-stressed, leading to a reduction in photosynthetic capacity [15, 19]. A decrease in C assimilation induces increased mobilization of C reserves to satisfy growth and maintenance metabolism. Although fine roots play a fundamental role in the uptake of water and nutrients, their absence due to lifting does not preclude water and nutrient uptake [7]. Therefore, in the case of young bare-root seedlings transplanted at the end of winter, two sources of nitrogen are to be considered for spring growth: N issued from reserves or newly absorbed.

If new root growth depends on new C and N assimilates, maintaining intact root and leaf systems at planting will be essential. On the contrary, if seedling regrowth is essentially under the dependence of C and N reserves, the availability of reserves may be crucial for a successful establishment in forest plantations. The importance of stored carbohydrates for new growth of lifted seedlings has often been underlined, but a close relationship between declining carbohydrate reserves and performance after transplanting was difficult to assess in practice [22].

Previous studies on Corsican pine, a species widely used for reforestation in southern Europe and for which substantial mortality occurs after transplanting, suggest that mechanisms specifically linked to both altered water and carbohydrate status are involved in transplanting stress [10, 14, 18]. However, precise relationships between water status, C and N reserves, new C and N assimilation, and previous events that concur to poor survival and low root growth after lifting, remain to elucidate.

Lifting and transplanting cause fine root damage. This stress can negatively impact C, N, and water uptake and then alter root regrowth. The major goal of the present study was to test the hypothesis of a relationship between regrowth and water, C and

N status of Corsican pine seedlings after lifting and transplanting. To test this hypothesis, we combined information given by total C and N content measurements, and by a dual ^{13}C and ^{15}N labelling of reserves of seedlings before lifting. By this way, we were able to assess whether lifting increased the importance of C and N derived from reserves *versus* new assimilates in supplying new root growth, and whether allocation of these elements between seedling components was changed by the altered root:shoot ratio. Effects of lifting were also assessed through concomitant measurements of carbohydrates, growth, gas exchange and water status of seedlings. Two main questions were addressed:

- Is the low – or the absence of – new root growth, induced by lifting, linked to altered water status and/or to reduced C and/or N availability?
- Does new growth depend primarily on C or N reserves or on new assimilates?

2. MATERIALS AND METHODS

2.1. Plant material

Two hundred, one-year-old seedlings of Corsican pine (*Pinus nigra* Arn. ssp. *laricio* var. *Corsicana*), were obtained in October 1995 from the nursery Robin (Saint-Laurent-du-Cros, Hautes-Alpes, S-E France). Seedlings were planted in 400 cm³ parallelepipedal plastic containers filled with sphagnum peat, and kept in a glasshouse at INRA Champenoux (N-E France), in which temperature was maintained above 8 °C. Seedlings were regularly watered with deionized water. After two months, 70 seedlings were randomly selected and submitted to a dual ^{13}C and ^{15}N long term labelling.

2.2. Labelling overview

Seedlings were submitted for one month to a dual $^{13}\text{CO}_2$ and $^{15}\text{NH}_4^{15}\text{NO}_3$ labelling. Seedlings were placed in a controlled climatic chamber (Vötsch VTPH 5/1000, Germany) operating as a semi-closed system and designed for $^{13}\text{CO}_2$ labelling [29]. Each pot was supplied with 10 mL of a 20 mM solution of $^{15}\text{NH}_4^{15}\text{NO}_3$ (99 atom% ^{15}N ; Eurisotop, Gif-sur-Yvette, France). Seedlings were exposed for 12 h twice a week over the one-month period to a $^{13}\text{CO}_2$ -enriched air (4 atom% ^{13}C) under a constant CO_2 concentration of 430 $\mu\text{mol mol}^{-1}$ air. Chamber temperature was 20 ± 1 °C, and relative humidity was 97%. Three high-pressure sodium vapour discharge lamps (Philips Electronics N.V., SONT, Amsterdam, The Netherlands) provided a photosynthetic photon flux density of 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at seedling level. Seedlings were returned into the greenhouse between the labelling cycles.

2.3. Experimental lifting and culture conditions after transplanting

At the end of the labelling experiment (day 0), root systems were gently washed to eliminate the ^{15}N labelled solution. Then, 27 seedlings had their root tips removed to simulate a nursery lifting. Simultaneously, the root system of 30 seedlings was kept intact, and these seedlings served as a control. After lifting, seedlings were transplanted into minirhizotrons (30 × 3 × 70 cm³) with a transparent side allowing root observations [26], filled with a fertilised sphagnum peat (TSK® 2 Instant, Floragard product; N: 300 to 450 mg L⁻¹, P₂O₅: 200 to 350 mg L⁻¹, K₂O: 350 to 600 mg L⁻¹) and irrigated every second day

to maintain field capacity throughout the experiment. Seedlings were grown for 41 days in a phytotron chamber (Weiss Technik, type 16 Sp, Resikirchen, Germany) under an ambient CO₂ concentration C_a = of 416 ± 10 µmol mol⁻¹. Temperature was 20/15 °C ± 1 °C (day/night) and relative humidity was 65/90 ± 5% (day/night). Thirty-six high-pressure sodium lamps (215 W, VHO, Sylvania Osram, GmbH, USA) provided a photosynthetic photon flux density of 350 µmol m⁻² s⁻¹ at the seedling level during the photoperiod (14 h).

2.4. Growth measurements

The new root length was measured weekly after transplanting and bud development was assessed according to a six level scale: dormant bud (0); swelled bud (1); appearance of new needles under scales (2); needles emerging from scales (3); unfolding of needles (4); needles expanded (5); needles expanded and starting stem elongation (6).

2.5. Water status and gas exchange measurements

Predawn needle water potential (Ψ_{wp}) was determined two days after transplanting, then weekly, on one needle per seedling using a Scholander pressure chamber. Gas exchange measurements were made in the climatic chamber 5 h after the beginning of the photoperiod, with a portable gas exchange measurement system (LI-6200, Li-Cor, Inc., Lincoln, NE, USA). Carbon dioxide assimilation rate (A, µmol m⁻² s⁻¹), needle conductance to water vapour (g, mmol m⁻² s⁻¹) and needle intercellular CO₂ concentration (C_i, µmol mol⁻¹) were calculated by means of classical equations [30]. At the end of the experiment, seedlings were harvested and their projected needle surface area was measured with a leaf area meter (Delta-T Devices, Cambridge, UK).

2.6. Sampling

The different sampling dates were: at transplanting, just after the labelling period (day 0), at the beginning of root regeneration (day 13), and on days 27 and 41. For each date, seven intact and nine lifted seedlings were sampled and separated into one-year-old needles, stem, old roots, new roots, and new shoots. In addition, three unlabelled seedlings were sampled on each sampling date to determine natural abundance of ¹³C and ¹⁵N in each seedling component. Each plant component was separately and quickly frozen in liquid nitrogen, freeze-dried, weighed and ground to a fine homogeneous powder with a laboratory mill (Retsch MM200, 42781 Haan, Germany). Samples were further stored at -20 °C until isotopic measurements and carbohydrate analyses.

2.7. Carbohydrate analyses

Soluble sugars were extracted using a method [8] based on differential polarity properties of solvents to separate soluble compounds from other biochemical ones. An aliquot of 3 to 8 mg of lyophilised dry matter was added to a ternary mixture (CHCl₃, H₂O, CH₂OH; 12/5/3; V/V/V) and incubated for ½ h at room temperature. The polar supernatant was kept and the extraction procedure was repeated two times to deplete the pellet in soluble compounds. Starch extraction was realised on the residue by incubation at 100 °C with NaOH (0.02N), then at 50 °C in presence of amyloglucosidase to hydrolyse starch in glucose molecules. Starch and soluble sugars (glucose, fructose and sucrose) were transformed in equivalent glucose with the help of two enzymes (β fructofuranosidase and hexokinase), and then measured by an enzymatic method [5], the principle of which being a stoichiometric relation existing between NADPH and the glucose content of the assay. Soluble sugars (glucose + fructose + sucrose) and starch were expressed in g C per 100 g C of tissue and their sum named total non-structural carbohydrates (TNC) in the text.

2.8. Isotopic analyses and calculations

Total C and N concentrations and ¹³C/¹²C or ¹⁵N/¹⁴N isotopic ratios of plant tissues were measured after combustion of aliquots of plant tissues in an elemental analyser (NA 1500 NCS, Carlo Erba, Milan, Italy) coupled with a mass spectrometer (VGA Optima, Fisons Micromass, England) at the Institut de Biotechnologie des Plantes (University Paris XI, Orsay, France). Calculations of isotopic enrichment and of allocation patterns of labelled and unlabelled elements were based on measured C and N isotopic compositions of the different seedling components at the end of labelling and during regrowth [4, 23]. For each sampling date, measurements of ¹³C and ¹⁵N isotopic abundances were made on the corresponding components of three unlabelled seedlings to determine natural ¹³C and ¹⁵N atom % background. For simplicity, variables related to C only are shown hereafter. Corresponding variables can be defined for N by substituting ¹³C, ¹²C and C with ¹⁵N, ¹⁴N and N, respectively.

– Isotopic abundance in atom % for carbon (A_C%) is defined as:

$$A_C \% = \frac{{}^{13}\text{C}}{{}^{13}\text{C} + {}^{12}\text{C}} \times 100$$

– Atom % excess is defined as the difference between the isotopic abundance of a given seedling component following administration of the ¹³C or ¹⁵N tracers (A_C labelled %), and the ¹³C or ¹⁵N natural abundance measured for the same component of unlabelled seedlings (A_C, unlabelled %):

$${}^{13}\text{C}_{\text{excess}} \% = (A_{\text{C, labelled}} \% - A_{\text{C, unlabelled}} \%) \times 100.$$

Isotopic abundance for C (A_C%) of each seedling component between the end of the labelling period (day 0) and the last day of the chase period (day 41) depended on a proportion of labelled C incorporated before transplanting (X_C; old C) and of unlabelled C assimilated after transplanting during the period of regrowth (Y_C; new C) with X_C + Y_C = 1:

$$A_{\text{C, labelled component}} \% = X_{\text{C}} (A_{\text{C, labelled seedling}} \% \text{ day 0}) + Y_{\text{C}} (A_{\text{C, unlabelled seedling}} \%)$$

where X_C, the proportion of old C, was estimated for a given seedling component as:

$$X_{\text{C}} = \frac{A_{\text{C, labelled component}} \% - A_{\text{C, unlabelled component}} \%}{A_{\text{C, labelled seedling, day 0}} \% - A_{\text{C, unlabelled seedling}} \%}$$

The abundance for ¹³C or ¹⁵N of labelled seedlings on day 0 (A_{C, labelled seedling, day 0} %) is assumed to correspond to C or N available for remobilisation at day 0. We did not assess such values but assumed that they were equal to those of bulk plant material [23].

Total content of C (Q_C) comprised old C (Q_{C, old}, incorporated before day 0) and new C (Q_{C, new}, incorporated after day 0) contents [4]:

$$Q_{\text{C}} = Q_{\text{C, old}} + Q_{\text{C, new}}$$

where

$$Q_{\text{C, old}} = X_{\text{C}} \times Q_{\text{C}}$$

with at day 0

$$Q_{\text{C}} = Q_{\text{C, old}}$$

and

$$Q_{\text{C, new}} = (1 - X_{\text{C}}) \times Q_{\text{C}}$$

2.9. Statistics

Two-way ANOVA (GLM procedure; SAS Institute Inc. 1989) followed by the Student-Newman-Keuls test were used to assess the effects of time and lifting on the different variables.

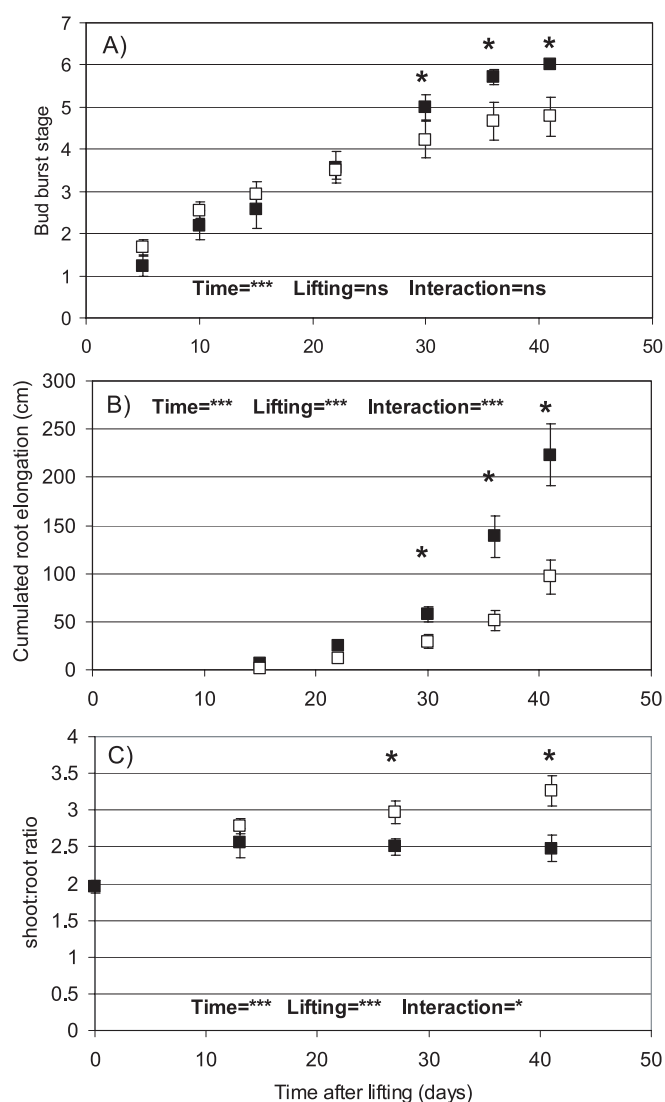


Figure 1. Changes after transplanting in bud burst (A), root elongation (B) and shoot:root ratio (C) of intact or lifted one-year-old Corsican pine seedlings grown in a controlled climatic chamber for 41 days. Mean values \pm SE ($n = 7$ to 9). (\square) lifted seedlings; (\blacksquare) intact seedlings. Bud development was assessed according to a six level scale: dormant bud (0); swelled bud (1); appearance of new needles under scales (2); needles emerging from scales (3); unfolding of needles (4); expanded needles (5); needles expanded and starting stem elongation (6). The different sampling dates were: at transplanting (day 0), at the beginning of root regeneration (day 13), and on days 27 and 41. The significance of the effects of lifting and time and their interaction are indicated for the different variables; ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3. RESULTS

3.1. Effects of lifting on bud break, new root elongation, and gas exchange

Bud break occurred immediately after transplanting and progressed regularly in intact and lifted seedlings (Fig. 1A). After 3 weeks, bud development of lifted seedlings had significantly fallen behind that of intact ones.

New roots appeared about 15 days after transplanting and grew exponentially in intact and lifted seedlings (Fig. 1B). Early, new root elongation was substantially lower in lifted seedlings than in intact ones.

The shoot: root ratio of lifted seedlings increased with time while that of intact seedlings increased until the 2nd week and then stabilized (Fig. 1C).

Lifting affected neither the C_i/C_a ratio nor Ψ_{wp} (Figs. 2B and 2D) but decreased both A and g (Figs. 2A and 2C).

3.2. Changes in total C and N after transplanting

Lifting caused significant changes in C concentration neither in seedlings nor in their components (results not shown). A significant increase with time was observed in N concentration of old and new roots as well as at whole seedling level (Tab. I). Conversely, N concentration in new shoots decreased with time. Lifting did alter N concentration neither in seedlings nor in their components. Time or lifting effects on C:N in the different seedling components were inversely related to those in N concentration.

Total carbon content of seedlings increased significantly with time, whereas the C content of needles and of old roots remained unaltered (Fig. 3 and Tab. II). With the exception of needles, lifting decreased C accumulation, particularly in old stem and old roots (Fig. 3 and Tab. II). Carbon accumulated in new components with time but lifting obviously restrained this accumulation.

Nitrogen content of seedlings and of their components, with the exception of needles, increased with time (Fig. 4 and Tab. II). Lifting caused a decrease in N content of whole seedlings and of old roots (Fig. 4 and Tab. II). In contrast, lifting had no effect on N content of needles and old stem. The N content of new components, and particularly new roots, was significantly decreased by lifting.

3.3. Characterisation of C and N sources for new growth

The increase with time of total C content of whole seedlings arose from new C accumulation, the old C content remaining quite stable (Fig. 3). Lifting lowered new C accumulation (Tab. II).

Old C content of old components was unchanged with time but lifting decreased it in old stem and old roots (Fig. 3 and Tab. II). New C content accumulated in pre-existing components with time without any lifting effect.

Lifting and time interacted and altered significantly new C accumulation in growing seedling components. New shoots grew exponentially after transplanting and accumulated rapidly both old and new C. On day 41, new shoots were mainly made with new C (Fig. 3 and Tab. II). Lifting altered significantly growth of new shoots, ending in a marked decrease in new C accumulation. New roots grew less than new shoots and also accumulated old C, with an increasing contribution of new C with time. Lifting decreased significantly new C accumulation in new roots.

As for C, the N content of seedlings increased with time due to the accumulation of new N, the old N content remaining quite stable (Fig. 4 and Tab. II). Lifting lowered the new N accumulation.

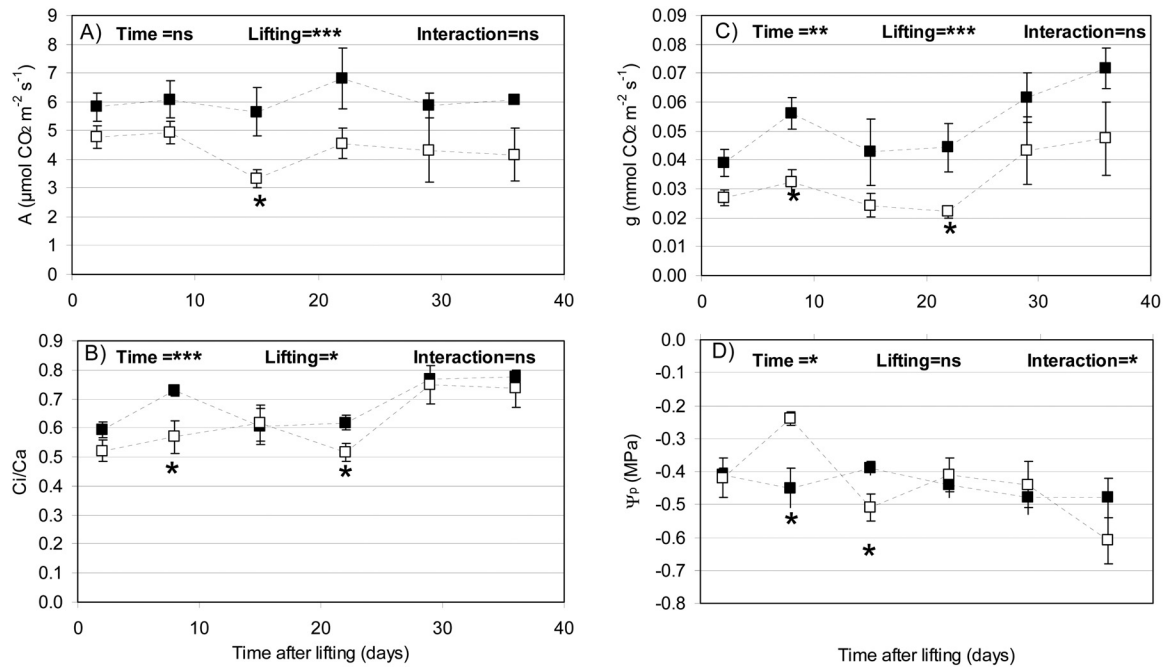


Figure 2. Changes after transplanting in net assimilation rate (A), Ci/Ca (B), stomatal conductance (C) and predawn water potential (D) of needles of intact or lifted one-year-old Corsican pine seedlings grown in a controlled climatic chamber for 41 days. Mean values \pm SE ($n = 7$ to 9). (\square) lifted seedling; (\blacksquare) intact seedling. The different sampling dates were: at transplanting (day 0), at the beginning of root regeneration (day 13), and on days 27 and 41. The significance of the effects of lifting and time and their interaction are indicated for the different variables; ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table I. Two-way variance analysis (ANOVA) for nitrogen concentration and C:N ratio of the various components of intact or lifted one-year-old Corsican pine seedlings grown after transplanting in a controlled climatic chamber for 41 days. Each value corresponds to a mean \pm SE ($n = 7$ to 9). The significance of the effects of lifting and time and their interaction are indicated for the different variables; ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Time	0			13		27		41		ANOVA		
	Intact	Intact	Lifted	Intact	Lifted	Intact	Lifted	Intact	Lifted	Time (T)	Lifting (L)	T \times L
N concentration %												
Needles	1.41 \pm 0.05	1.14 \pm 0.07	1.21 \pm 0.07	1.14 \pm 0.06	1.10 \pm 0.05	1.23 \pm 0.08	1.20 \pm 0.05	ns	ns	ns	ns	ns
Old stem	0.61 \pm 0.03	0.70 \pm 0.07	0.63 \pm 0.04	0.79 \pm 0.07	0.65 \pm 0.04	0.77 \pm 0.07	0.93 \pm 0.05	**	ns	*	ns	*
Old roots	0.87 \pm 0.06	1.12 \pm 0.11	1.16 \pm 0.06	1.20 \pm 0.12	1.16 \pm 0.07	1.27 \pm 0.05	1.25 \pm 0.03	***	ns	ns	ns	ns
New shoot	2.80 \pm 0.16	2.21 \pm 0.11	2.23 \pm 0.07	1.41 \pm 0.03	1.38 \pm 0.04	1.51 \pm 0.10	1.74 \pm 0.18	***	ns	ns	ns	ns
New roots	2.05 \pm 0.12	2.45 \pm 0.29	2.05 \pm 0.01	2.71 \pm 0.20	2.86 \pm 0.09	2.66 \pm 0.18	2.96 \pm 0.13	***	ns	ns	ns	ns
Whole seedling	1.08 \pm 0.05	1.07 \pm 0.07	1.09 \pm 0.05	1.11 \pm 0.07	1.05 \pm 0.04	1.22 \pm 0.07	1.22 \pm 0.07	*	ns	ns	ns	ns
C:N ratio												
Needles	35.5 \pm 1.4	42.8 \pm 2.4	42.0 \pm 2.1	42.7 \pm 2.2	44.3 \pm 2.8	40.4 \pm 2.4	41.4 \pm 1.7	ns	ns	ns	ns	ns
Old stem	82.0 \pm 4.4	71.6 \pm 6.0	77.6 \pm 4.0	63.5 \pm 5.7	74.3 \pm 4.8	66.0 \pm 5.3	53.2 \pm 2.8	*	ns	ns	ns	ns
Old roots	57.2 \pm 3.9	44.7 \pm 2.6	42.4 \pm 2.2	43.7 \pm 3.9	42.4 \pm 2.3	39.2 \pm 1.6	40.7 \pm 1.3	***	ns	ns	ns	ns
New shoot	18.0 \pm 1.2	21.9 \pm 0.9	22.0 \pm 0.7	34.0 \pm 1.2	35.8 \pm 1.3	33.0 \pm 2.5	29.5 \pm 2.4	***	ns	ns	ns	ns
New roots	22.7 \pm 1.3	19.8 \pm 2.4	22.0 \pm 0.3	17.5 \pm 1.6	15.7 \pm 0.5	17.8 \pm 1.3	15.5 \pm 0.6	***	ns	ns	ns	ns
Whole seedling	46.0 \pm 2.0	45.8 \pm 2.4	45.3 \pm 1.8	44.6 \pm 2.7	46.2 \pm 2.0	40.4 \pm 2.4	40.9 \pm 1.5	*	ns	ns	ns	ns

Old N content of pre-existing components was significantly altered by time and particularly, decreased markedly from day 0 to day 13 in needles (Fig. 4 and Tab. II). No modulation by

lifting was observed, except in old roots exhibiting a significant decrease in old N content in response to lifting. New components first incorporated old N, and new shoots incorporated

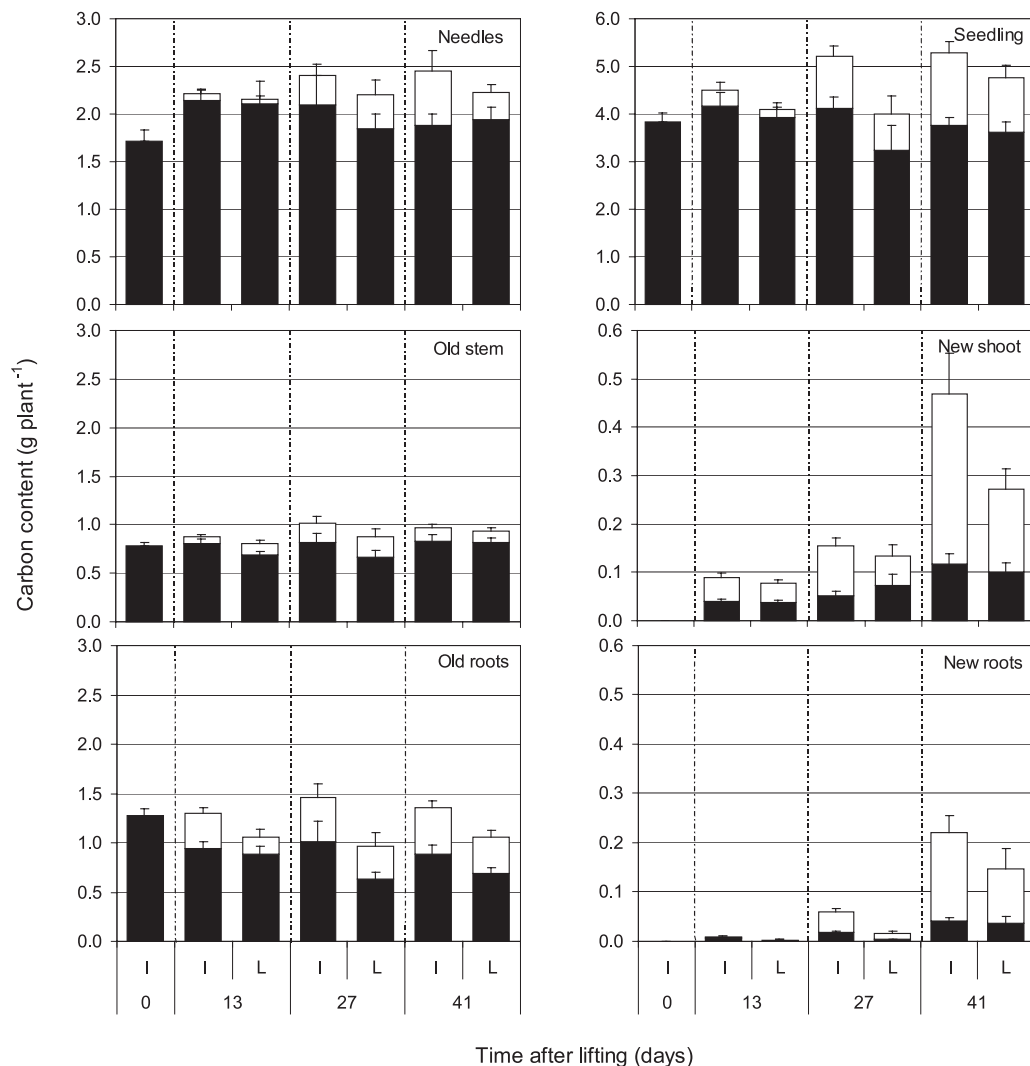


Figure 3. Changes in content of old (■) and new (□) carbon in the various components of intact (I) or lifted (L) one-year-old Corsican pine seedlings grown in a controlled climatic chamber for 41 days. Mean values \pm SE ($n = 7$ to 9). The different sampling dates were: at transplanting (day 0), at the beginning of root regeneration (day 13), and on days 27 and 41.

more intensively old N than new roots. Lifting decreased old N accumulation in new roots only.

New N content increased with time in all components but accumulated earlier and mostly in needles (Fig. 4 and Tab. II). Lifting did not alter significantly this accumulation in pre-existing components. Lifting reduced markedly new N accumulation in new shoots. At the end of the experiment, new shoots were mainly constituted by old N. In contrast, new roots were mainly made with new N. However, lifting decreased new N accumulation in new roots.

3.4. Changes in C and N partitioning after transplanting

At transplanting, about 45% of total C in intact seedlings was found in needles, 33% in old roots, and 21% in old stem (Fig. 5 and Tab. II). Total C partitioning to needles was stable with

time but was increased by lifting (+12%). Partitioning of total C to the stem (20%) was unaffected by lifting and time. Partitioning of total C to old roots decreased with time and lifting. Partitioning of total C to new components increased with time. Forty-one days after transplanting, new roots and new shoots of intact seedlings contained ca. 4 and 9% of total seedling C, while these proportions were 2 and 6% in lifted ones, respectively (Fig. 5 and Tab. II). Lifting significantly decreased the partitioning of total C to new roots only.

Old C (45%) and new C (0 to 30%) partitioning to needles increased with time without any lifting effect (Fig. 5 and Tab. II). Lifting did not alter old and new C partitioning to the stem. Old C partitioning to old roots decreased from 30 to 20% with time with no lifting effect, whereas, new C partitioning first strongly increased to 60% (day 13) and then decreased to 30% (day 41). Lifting decreased by 2 new C partitioning to old roots at day 13. Old and new C partitioning to new roots significantly

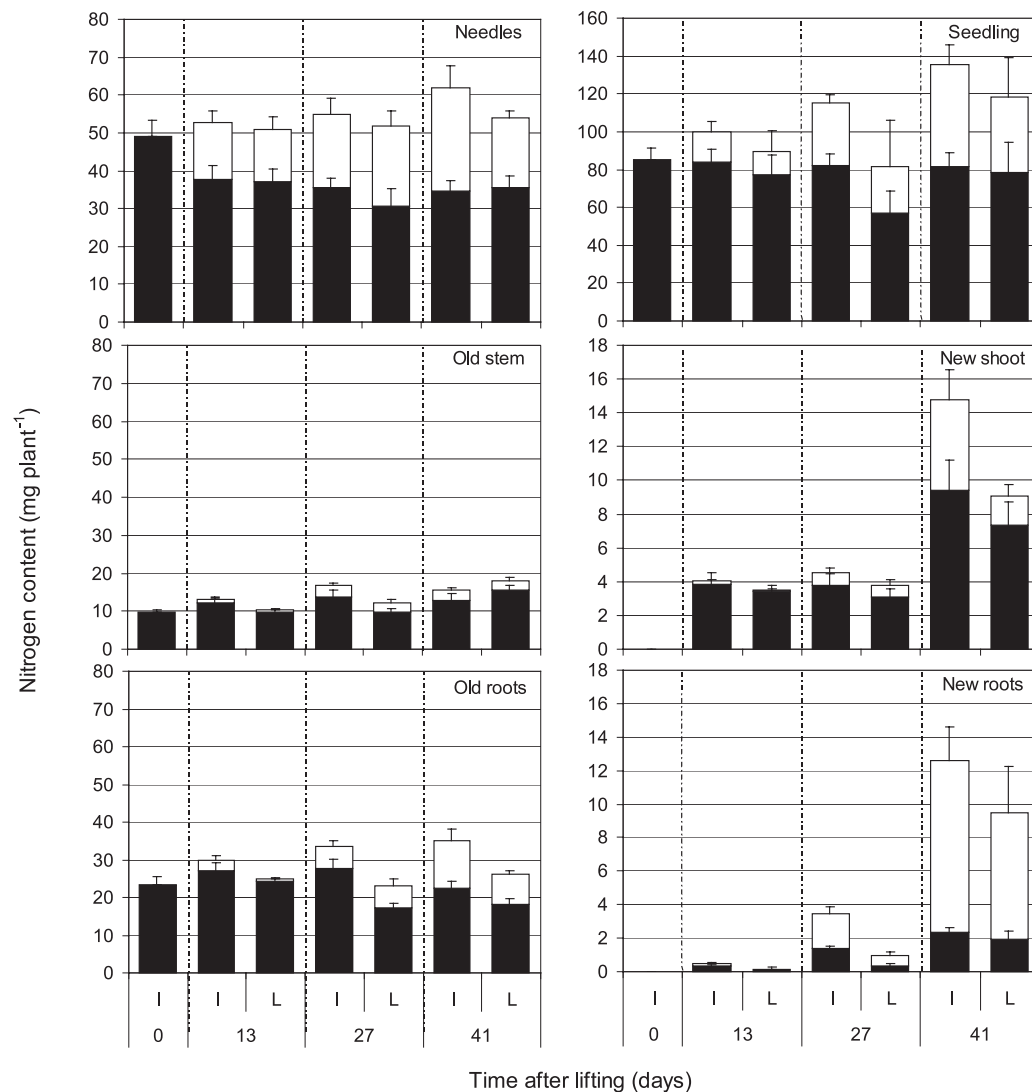


Figure 4. Changes in content of old (■) and new (□) nitrogen in the various components of intact (I) or lifted (L) one-year-old Corsican pine seedlings grown in a controlled climatic chamber for 41 days. Mean values \pm SE ($n = 7$ to 9). The different sampling dates were: at transplanting (day 0), at the beginning of root regeneration (day 13), and on days 27 and 41.

decreased with time and lifting (Fig. 5 and Tab. II). Old and new C partitioning to new shoots increased with time with no significant effect of lifting.

As for C, total N was partitioned by decreasing order to needles (58%), old roots (27%) and old stem (12%) (Fig. 5 and Tab. II). Total N partitioning to old roots slightly increased with time and decreased in needles. Lifting did not modify N partitioning to needles, but decreased N partitioning to old roots (Fig. 5 and Tab. II). Lifting decreased significantly the partitioning of total N to new roots whereas the partitioning to new shoots was not affected. Forty-one days after transplanting, new roots and new shoots of intact seedlings contained ca. 9 and 11% of total seedling N, while these proportions were 5 and 8% in lifted seedlings (Fig. 5 and Tab. II).

Partitioning of old N to needles (60%, day 0) was decreased by about 33% (day 41) with time (Fig. 5 and Tab. II). Partitioning of new N to needles increased strongly the two first weeks

after transplanting, representing about 90% of total seedling N. Then, it decreased to about 50% without any lifting effect. Partitioning of old N to old stem increased with time (from 10 to about 20%) without effect of lifting, whereas in old roots it decreased markedly from 35 to 20% in response to lifting only.

New N partitioning to old roots and to old stem increased with time (20% and 5%, respectively) without any lifting effect (Fig. 5 and Tab. II). Partitioning of old and new N to new components increased with time. Lifting decreased old N partitioning to new roots only.

3.5. Changes in carbohydrate concentration after transplanting

Total non-structural carbohydrate concentration of seedlings increased from 10 to 15% during the two first weeks and then stabilised (Fig. 6). This increase was mainly due to starch accumulation in needles (from 6 to 10%). Lifting slightly but

Table II. Effects of lifting and time on changes in content and partitioning of total, old and new elements (C and N) in the various components of intact or lifted one-year-old Corsican pine seedlings grown after the labelling period in a controlled climatic chamber for 41 days. Labelling period lasted 1 month and was followed by a chase period of 0, 13, 27 or 41 days. Each value corresponds to a mean \pm SE ($n = 7$ to 9). The significance of the effects of lifting (L) and time (T) and their interaction (T \times L) were assessed by a two-factorial variance analysis (ANOVA), and are indicated for the different variables; ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ANOVA	Needles			Old stem			Old roots			New shoot			New roots			Whole seedling		
	T	L	T \times L	T	L	T \times L	T	L	T \times L	T	L	T \times L	T	L	T \times L	T	L	T \times L
Total C content	ns	ns	ns	***	*	ns	ns	**	ns	***	*	ns	***	**	*	**	*	ns
Total N content	ns	ns	ns	***	ns	ns	**	**	ns	***	*	ns	***	**	ns	***	*	ns
Old C content	ns	ns	ns	ns	*	ns	ns	*	ns	***	ns	ns	***	*	ns	ns	*	ns
Old N content	*	ns	ns	*	ns	ns	*	**	ns	***	ns	ns	***	***	ns	ns	ns	ns
New C content	**	ns	ns	***	ns	ns	***	ns	ns	***	**	*	***	**	*	***	ns	ns
New N content	***	ns	ns	***	ns	ns	***	ns	ns	***	*	*	***	*	ns	**	*	ns
Partitioning total C	ns	**	ns	ns	ns	ns	***	***	ns	***	ns	ns	***	***	*	—	—	—
Partitioning total N	***	**	ns	*	ns	*	**	*	ns	***	ns	ns	***	***	*	—	—	—
Partitioning old C	*	ns	ns	ns	ns	ns	***	ns	ns	***	ns	ns	***	*	ns	—	—	—
Partitioning old N	***	*	ns	***	ns	*	***	*	ns	***	ns	ns	***	***	*	—	—	—
Partitioning new C	**	ns	ns	**	ns	ns	***	ns	ns	***	ns	ns	***	***	ns	—	—	—
Partitioning new N	***	ns	ns	***	ns	ns	***	ns	ns	***	ns	ns	***	***	ns	—	—	—

significantly reduced this increase at the seedling level. TNC concentration of old stem was quite stable (about 10%) whereas starch concentration decreased with time and no effect of lifting was observed (Fig. 6). Concentration of TNC in old roots decreased strongly (from 15 to 5%) with time due to the decrease of both starch (from 9 to 2%) and soluble sugar (from 5 to 3%) concentrations. Regardless of lifting, concentration of TNC in new components reached 10 to 15% two weeks after planting then decreased in new roots and new shoots to 10 and 5%, respectively. These changes in TNC concentration in young growing components mainly involved soluble sugars (about 6%).

4. DISCUSSION

After transplanting, lifted Corsican pine seedlings exhibited reduced new growth and alterations in their C and N assimilation as compared to control seedlings. Lifting decreased carbon assimilation and stomatal conductance but $C_i:C_a$ ratio and predawn water potential remained unaltered. This last point led us to hypothesize that the decrease in net CO_2 assimilation rate in Corsican pine seedlings in response to lifting, implied both stomatal and metabolic processes. The photosynthetic activity in plants is generally strongly correlated with their N concentration even if the relationship between N concentration and CO_2 assimilation capacity is weaker in conifers than in other species due to lower rates of photosynthesis and a smaller range of N concentration than in non-coniferous species [25, 32]. The proportion of leaf N found in Rubisco is known to vary between 6–20% in evergreen conifers [12, 28] but, in tree seedlings the proportion of N allocated to Rubisco has been found to be independent of N supply [33]. In our experiment, newly assimilated N increased less in lifted versus control seedlings. However, total N content of needles of lifted seedlings was unaltered by the decrease in N uptake. Besides, new N content of needles of

lifted seedlings increased significantly. This result led us to hypothesize both an increased export of old N from needles in response to lifting, and an increased import of currently assimilated N. Such changes in N redistribution could be partly linked to the fact that the removal of new roots, by temporarily precluding efficient N uptake, imposes that lifted seedlings rapidly restore new roots through the utilisation of C and N reserves. Rubisco being considered as a major form of N storage in plants [32], should be remobilised more intensively in needles of lifted than of control seedlings. The observed decrease in net assimilation could be related to the increased turnover of Rubisco in response to lifting.

No water stress was observed in our experimental conditions as shown by the unaltered predawn water potential of needles. Further confirmation of a lack of alteration of hydraulic conductivity in lifted seedlings could be drawn by concomitant water potential and gas exchange measurements [16]. However, the fact that N uptake decreased but did not cease after lifting in our experiment, suggests that root systems of lifted Corsican pine seedlings remained able to acquire water and nutrients after transplanting. This behaviour was already observed for loblolly pine seedlings [7] submitted to a restriction of their root system. Probably, a prolonged exposition to ambient air before transplanting or a cold storage are more susceptible to induce drastic plant desiccation [11, 13, 14] than lifting alone if a satisfactory water supply is provided after transplanting. However, even if lifting only caused a small loss of biomass for the root system, physiological status of transplanted seedlings was significantly damaged. Indeed, a marked decrease in bud break, root and shoot growth occurred early and remained visible almost two months after transplanting. However, shoot growth was less affected than root growth as previously shown for Douglas fir [16]. A water limitation could be indirectly generated later in the growing season if the growth unbalance generated

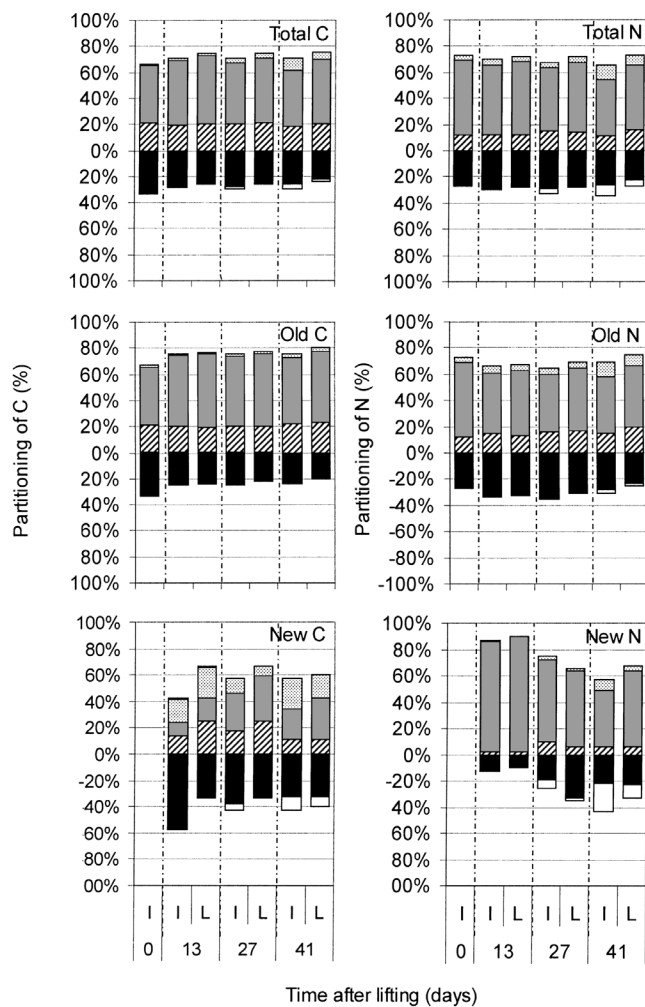


Figure 5. Changes in partitioning of total, old, and new carbon and nitrogen in various components of intact or lifted one-year-old Corsican pine seedlings grown in a controlled climatic chamber during 41 days. Mean values \pm SE ($n = 7$ to 9). New roots (□); old roots (■); old stem (▨); needles (▩); new shoots (□). The different sampling dates were: at transplanting (day 0), at the beginning of root regeneration (day 13), and on days 27 and 41.

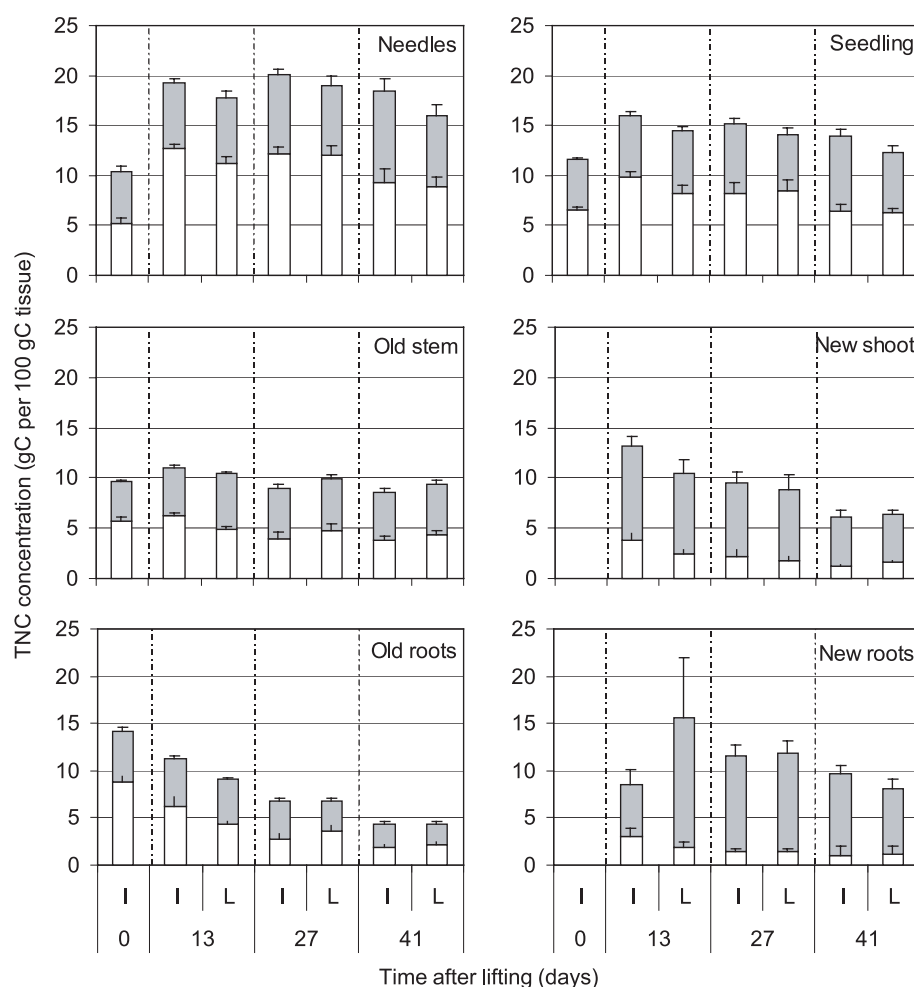
by lifting persists. Considering that other usual post-cultural operations such as short-term exposure to ambient conditions, will also concur to decreased root growth of Corsican pine seedlings after transplanting [14], it is likely that increased water demand of the aerial system will be more difficult to satisfy and will jeopardize successful establishment of these seedlings.

Lifting ended early in a significant decrease in C acquisition. However, at the whole plant level, new C content of lifted seedlings was not significantly decreased compared to intact ones (Tab. II). These results led us to hypothesize that C loss by respiration could be lower in lifted seedlings than in intact ones, partly due to decreased growth. No marked imbalance in C:N ratio was generated in seedlings by lifting in our experimental conditions, due to a similar and slight decrease of these elements with lifting. Lifting, through a slight decrease in non-structural

carbohydrate concentration, also altered carbohydrate status of Corsican pine seedlings. Starch concentration increased strongly in needles the first days after transplanting. Then, starch was mobilised in response to increased growth needs and seedling concentration decreased from 10% at day 13 to 6% on day 41. No strong TNC decrease occurred in components with time except in old roots. It seems that decreased growth of lifted seedlings cannot be attributed directly to reduced availability of carbohydrates as already suggested for the same species [14].

Amounts of labelled C and N incorporated by Corsican pine seedlings before lifting did not decrease significantly with time but were redistributed for growth of new components, pointing to the absence (1) of consumption of C reserves for respiration and (2) of no important N release in the soil after lifting as previously demonstrated on *Quercus suber* saplings [6]. Few C reserves were mobilised in old stem that acted more as a sink than a source for C and N. Contrastingly, old roots and needles of Corsican pine cooperated to provide C and N for growth of new components. Old roots acted as a source of C reserves and needles as source of N reserves, about 50% of N of seedlings being located in them. Shoot growth began immediately after transplanting and was supported by old C as demonstrated both by its C composition and by the concomitant strong decrease in old C content, starch and soluble sugar concentrations in old roots. Nitrogen reserves of needles were also intensively mobilized the first weeks to support shoot growth. In contrast, new roots appeared 15 days later than new shoots, even in case of lifting, and used less C and N reserves than new shoots. Forty-one days after transplanting, new roots were mainly constituted by new C and N, while new shoots remained enriched in old N still representing about 64 (lifting) or 80% (intact) of its total N content. This result led us to hypothesize that shoot growth, by preferentially using C and N reserves, will be less penalized by a temporary decreasing availability of C and especially N than root growth which is more dependent on new assimilates. Besides, our results show that reduced root growth operated with no substantial decrease of TNC in seedlings as already reported in [14]. Consequently, N availability seems more limiting than C availability for root regrowth in Corsican pine seedlings. Lifting also significantly decreased the partitioning of newly acquired C and N to the new growing components, particularly for new roots. Precisely, new C and new N stayed more in old structures in lifted than in intact seedlings. In fact, the most important differences appear in N use for new growth. First, lifting ended up in alterations in the use of old C and N by new growing components. Second, the growing components used less new acquired C and N, ending in decreased growth. The most important sinks for new N were needles that restored the loss of old N occurring with time by new N, so that total N content remained unaltered in these components. In fact, after transplanting, needles attracted between 84 and 88% of total new N in seedlings the 13 first days and still between 43 and 58% after 41 days. This result implies that, contrarily to C, in Corsican pine seedlings, N was not firstly renewed in components (roots, stem) nearest of the absorption source.

In conclusion, our results point to the fact that a root system with intact fine roots is an important prerequisite for new growth of transplanted Corsican pine seedlings, even if lifting did not cause water stress or impeded N uptake. Our results suggest that regulations operate in seedlings at the needle level to



ANOVA	Needles		Old stem		Old roots		New shoot		New roots			Whole seedling	
	T	L	T	L	T	L	T	L	T	L	T × L	T	L
Sol. sugars	*	ns	*	ns	***	ns	**	ns	ns	ns	*	ns	ns
Starch	***	ns	*	ns	***	ns	**	ns	**	ns	ns	***	ns
TNC	***	ns	ns	ns	***	ns	**	ns	ns	ns	ns	***	*

Figure 6. Changes in total non-structural carbohydrate (TNC) concentration of various components of intact (I) or lifted (L) one-year-old Corsican pine seedlings grown in a controlled climatic chamber for 41 days. Each value is expressed in g C per 100 g C tissue, and is the average of 7 to 9 replicates \pm SE. Starch (■); Soluble sugars (□). The significance of the effects of lifting (L) and time (T) and their interaction (T \times L) are indicated for the different variables; ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. No interaction was found with the exception of new roots.

limit negative effects of decreased water and N entry, through decreased stomatal conductance and increased allocation of N. As a consequence, C assimilation, carbohydrate concentration, and N availability for new growing components were decreased in lifted seedlings. However, root growth was more penalised than shoot growth, probably due to delay in regrowth and to a greater dependence on new assimilates than new shoots, leading to a growth unbalance. This unbalance combined with other root injuries generated by horticultural practices can increase

strongly risks of degradation of water potential and C and N acquisition on the site of installation, and compromise successful plantation establishment in the field.

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