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FORESTRY

RESEARCH PAPER

The influence of N and P supply and genotype on N remobilization in containerized *Pinus radiata* plants

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Abstract

H.E. Bown, M.S. Watt, P.W. Clinton, and E.G. Mason. 2012. The influence of N and P supply and genotype on N remobilization in containerized *Pinus radiata* plants. Cien. Inv. Agr. 39(3): 505-520. A large proportion of the nitrogen (N) used in the current-year growth of the widely grown plantation species *Pinus radiata* D. Don is N that was stored in plant tissues the previous year. However, the extent to which an imbalance between levels of phosphorus (P) and N may change the capacity of plants to remobilize N is unknown. In this study, the N remobilization responses of four *P. radiata* genotypes to a factorial combination of N and P additions were assessed in containerized plants in a two-year greenhouse experiment. N supply was enriched with ^{15}N at 2.5 ‰ (labeled N) during the first year. Plants were then transferred to clean sand and grown for another year with ^{15}N at levels close to natural levels (0.3664899 atom percent ^{15}N, $\delta^{15}N$ 0.5115 ‰). Calculations of N storage and remobilization were based on the recovery of labeled N from new tissues during the second year of growth. Over the second year, N remobilization for the high-N high-P supply regime (953 mg N plant^{-1}) was five-fold that of the N remobilization for the low-N low-P supply regime (199 mg N plant^{-1}), with the unbalanced high-N low-P (422 mg N plant^{-1}) and low-N high-P (228 mg N plant^{-1}) treatments showing intermediate N remobilization. Sixty-five percent of the plant N content at the end of the first year of growth was remobilized during the second year in the high-N high-P supply treatment, compared to 42-48% for the other N and P addition treatments. The ratio of N uptake to N remobilization was greater for the high-N supply regimes compared to the low-N supply regimes, suggesting that trees may rely progressively more on remobilization than uptake as N fertility declines. Most N remobilization (77%) occurred during the spring-summer periods and coincided with the largest proportion of needle development (80%), suggesting that N remobilization was driven by sink-strength. Old foliage was the main source for internal cycling, while roots were the main sink. Faster-growing genotypes did not exhibit an enhanced capacity for N remobilization, suggesting that genetically induced growth performance is not explained by N internal cycling. In conclusion, trees growing with an abundant and balanced nutrient supply exhibited greater capacity for nitrogen remobilization than trees with N and/or P deficiencies however, faster-growing clones did not exhibit an enhanced capacity for N remobilization.

Key words: genotypes, nitrogen, nutrient remobilization, phosphorus, *Pinus radiata*.

Introduction

Plant nutritional stresses are commonly associated with nutrient imbalances rather than single-nutrient deficiencies (Reich and Schoettle, 1988; Marschner, 1995; Aerts and Chapin, 2000). The mechanisms leading to nutrient imbalances can be difficult to unravel because the factors affecting nutrient uptake and internal cycling are poorly understood (Proe *et al.*, 2000). Conifers rely

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heavily on their capacity for internal cycling of mobile nutrients for growth and survival (Millard and Proe, 1993; Nambiar and Fife, 1987; Nambiar and Fife, 1991; Proe *et al*., 2000). For the widely grown plantation species *Pinus radiata* D. Don, previous research shows that a large proportion of N (32-57%) and P (40-86%) in new tissues is sourced from remobilization of these nutrients from old tissues (Fife and Nambiar, 1982; Nambiar and Fife, 1987).

Nutrient remobilization in conifers has been determined by sequential sampling of needle masses and nutrient contents (nutrient budgets) and by using stable isotopes (Proe *et al*., 2000). Nutrient budgets underestimate the relative contribution of remobilization because uptake from the soil and remobilization cannot be determined separately (Mead and Preston, 1994; Proe and Millard, 1994; Proe *et al*., 2000). Stable isotopes, on the other hand, have been frequently and reliably used to partition and quantify nutrient uptake and remobilization. Such studies have shown that the mobile nutrients required for tree growth are obtained in similar quantities through two processes: current uptake and remobilization from old to new tissues (Proe *et al*., 2000). Nutrient remobilization has been shown for both N (Proe and Millard, 1994) and P (Proe and Millard, 1995), but investigations on the interactive influence of nitrogen and phosphorus supply on nitrogen remobilization in pines have not yet been undertaken. Although foliage seems to be the main source for nutrient remobilization in pines, the use of stable isotopes has also shown that woody tissues also contribute to nutrient remobilization in *Pinus contorta* (Mead and Preston, 1994). Nambiar (1987), using nutrient budgets, showed for *P. radiata* that foliage is an important source for N remobilization, but limitations in the technique restricted conclusions around the extent to which woody tissues contributed to N remobilization. To address this gap in knowledge, we used stable isotopes to determine whether labeled N could be traced in new woody tissues during the second year of plant growth.

Genetics is emerging as an important factor controlling internal nutrient efficiency and remobilization. For instance, Miller and Hawkins (2003) compared nitrogen uptake and utilization by slow- and fast-growing families of interior spruce in a greenhouse experiment using different nitrogen supply regimes. Individuals from the fast-growing families developed more rapidly and were more efficient in the utilization of internal nitrogen at all fertility levels, suggesting a potential for tree growth improvement based on nitrogen-use-efficiency traits. Similarly, Fife and Nambiar (1995) found large differences in the growth responses of *P. radiata* families to nitrogen additions, suggesting that nutrient remobilization capacity may at least partially account for the differences in growth among families.

Using large container-grown *P. radiata* clones, the aim of our study was to use ^{15}N to determine the interactive influences of N and P supply on N storage and remobilization in *P. radiata*. We also examined whether the contrasting growth patterns in the four clones could be attributed to variation in N-remobilization efficiency. The hypotheses for the study were that (i) N remobilization increases with N supply but is constrained by N and/or P imbalances, (ii) the contribution of woody tissues to N remobilization is minimal, and (iii) fast-growing clones exhibit greater capacity for N storage and remobilization than slow-growing clones, which would at least partially explain differential clonal growth performance.

Materials and methods

Plant material

Plant material was collected in a greenhouse experiment using four clones of *P. radiata* irrigated with a factorial combination of two levels of nitrogen additions (low-N=1.43 mol m^{-3} and high-N=7.14 mol m^{-3}, supplied as NH_4NO_3) and two levels of phosphorus additions (low-P=0.084

mol m^{-3} and high-P=0.420 mol m^{-3}, supplied as KH_2PO_4). Ingestad (1979) suggested that N should be provided at concentrations of 100 ppm (7.14 mol m^{-3}) and P at 13 ppm (0.420 mol m^{-3}) for optimum growth of *Pinus sylvestris* L. These concentrations were used for the high-N and high-P supply regimes, respectively. For the low-N and low-P treatments, one-fifth of the high-N and high-P addition rates, respectively, were used. Nitrogen additions for the low-N and high-N treatments were 62.8 mmol N and 314.1 mmol N per plant, respectively, over the duration of the experiment. Phosphorus additions in the low-P and high-P treatments were 3.69 mmol P and 18.47 mmol P, respectively, applied to each plant over the duration of the experiment. Additional nutrients were provided at the following concentrations: 0.51 mol m^{-3} K, 0.25 mol m^{-3} Ca, 0.41 mol m^{-3} Mg, 0.28 mol m^{-3} S, 12.53 mmol m-3 Fe, 0.45 mmol m^{-3} Zn, 0.47 mmol m^{-3} Cu, 7.28 mmol m^{-3} Mn, 0.073 mmol m^{-3} Mo, 18.50 mmol m^{-3} B, 0.85 mmol m^{-3} Cl, and 0.13 mmol m^{-3} Na, following Ingestad (1979).

One-year-old *P. radiata* cuttings from four clones (P26C2, P26C5, P08C9 and S11C3), were selected to represent a gradient in growth performance within a set of 400 genotypes planted in the Purokohukohu Experimental Basin on the central North Island of New Zealand (Beets *et al*. 2004). The "P" clones (P26C2, P26C5, P08C9) were from a population of trees that grow on pumice soil at Puruki in the central North Island and have a variety of nutrition-related upper and middle crown yellowing symptom scores, while the "S" clone (S11C3) was from a population of trees that grow on serpentine soil near Nelson on the northern South Island (Beets *et al*. 2004). One-year-old *P. radiata* cuttings from the four clones (hereafter called A, B, C and D) were raised at a nursery in SCION in Rotorua before being transplanted to 4.2 L pots containing approximately 5.5 kg of air-dry silica sand (<0.1% organic matter).

The plants were arranged in a completely randomized block design of three blocks, each of which included 48 plants (three replicate plants × four clones × four fertilizer regimes). Nutrient treatments were randomly allocated to the plants and applied for two years, from winter (June) 2004 to winter (June) 2006. All plants received 0.5 L of nutrient solution per week during the first year and that amount was doubled during the second year. All plants were daily supplied with water in excess of the plant requirements and were allowed to drain freely. The roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. at the beginning of the experiment. To inoculate the roots, 1 g of spores were mixed with 1 L of distilled water and 10 mL were applied to each plant, equivalent to 11.9 × 106 spores per plant. The presence of mycorrhizae was confirmed either by visual inspection of the roots or by the presence of fruiting bodies. Temperature in the greenhouse was thermostatically controlled to keep temperatures in the range of 5-32 °C, but was not further controlled within this range. Air temperature and solar radiation inside the greenhouse simulated the natural conditions in the outside environment.

Labeled N was applied as $^{15}NH_4{}^{15}NO_3$ enriched to 2.5 atom percent ^{15}N for one year, from June 2004 until the plants went dormant in winter (July) 2005, and then N was applied as NH_4NO_3 with ^{15}N at 0.3664899 atom percent ^{15}N ($\delta^{15}N$ 0.5115 ‰) during the second year, ending in June 2006.

Sequential harvests

At the end of the first year of growth, while plants were still dormant, one of the three replicate plants from each of the 48 treatment × clone × block combinations were randomly selected and harvested (Figure 1, indicated as Harvest 1). Following Millard and Proe (1992), all remaining trees were removed from their pots, the sand was carefully washed from the roots, and the trees were transferred into 42 L pots with clean sand. During the second year, one of the two remain-

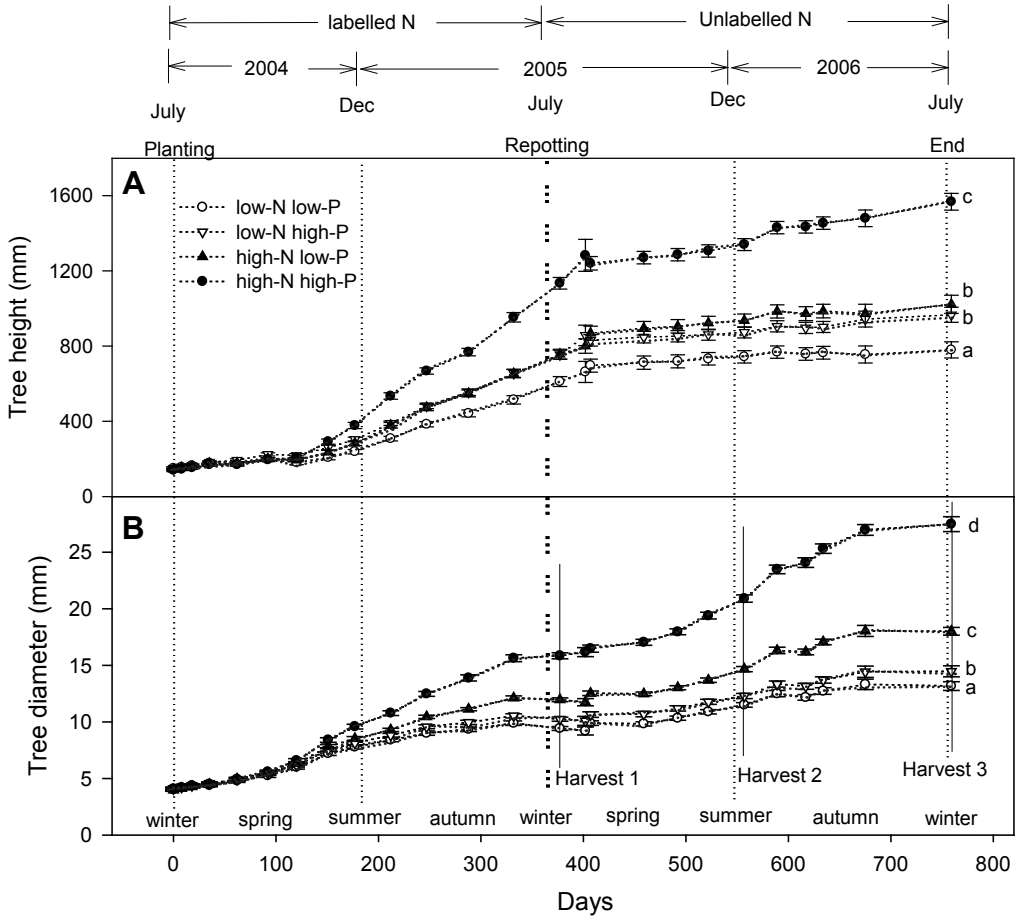

Figure 1. Plant development over 24 months showing (A) tree heights and (B) basal diameters across different nutrient treatments: low-N low-P (open circles), low-N high-P (downward-facing open triangles), high-N low-P (upward-facing filled triangles) and high-N high-P (filled circles). Figure (B) shows times of harvest at months 12, 18 and 24. Letters at the far right of the each curve in (A) and (B) indicate significant differences in tree height and diameter growth at the end of the experiment ($P \leq 0.05$).

ing replicates from each treatment was randomly selected and harvested at month 18 (Harvest 2), with the remaining 48 plants harvested at month 24 (Harvest 3) (Figure 1).

During all destructive harvests, the shoots were separated into stems, branches and foliage from the first and second year of plant growth. The bulk root system was collected and the sand was sieved to recover all visible roots. The sieved sand was thoroughly mixed and weighed and a 2.5 kg subsample was taken to recover the remaining roots by flotation. The roots were washed and separated into fine roots (≤2 mm), coarse roots (>2 mm), and below-ground stems. The fine roots were separated into first- and second-year growth during the harvests at ages 18 and 24 months. First-year root growth was defined as the roots located within the confines of the initial 4.2 L pots, while second-year root growth was defined as the roots outside of the 4.2 L pots but within the remaining volume of the 42 L large pots . All plant components were oven-dried to constant mass at 70 °C.

Nitrogen isotopic composition

Oven-dried samples from foliage, stems and roots from first- and second-year growth from all plants and harvests were ground to a fine powder using a rotary mill (Thomas-Wiley Laboratory Mill, Model 4, Philadelphia, PA., USA) and a vibratory ball mill (Retsch MM-2000, Haan, Germany). There were 144 samples from the first harvest (4 treatments × 4 clones × 3 blocks × 3 components: roots, stems and foliage); a total of 288 samples from either the second and third harvest (4 treatments × 4 clones × 3 blocks × 3 components: roots, stems and foliage x 2 ages: current and old); and an additional 45 samples including 8 samples from cuttings at the time of planting (4 clones × 2 cuttings), 32 samples from litter (4 clones × 4 treatments × 2 harvests), and 5 samples as standards, for a total of 765 samples. For all samples, total N and its isotopic composition ($\delta^{15}N$) was determined using a mass spectrometer (Stable Isotope Laboratory at the University of Waikato, New Zealand). The δ values were calculated as: $\delta\ (‰) = [(R_{sp} / R_{st}) - 1] \times 1000$, where R_{sp} and R_{st} are the $^{15}N/^{14}N$ ratios of the sample and the $^{15}N/^{14}N$ ratios of the standard (N^2 in air), respectively.

Calculation of nutrient storage and remobilization

A ^{15}N recovery and mass-balance approach was used to determine N remobilization for each tree. Tissue ^{15}N content was calculated as the product of tissue mass, tissue N concentration and tissue ^{15}N atom percent (excess). Tissue ^{15}N atom percent (excess) was calculated as the difference between the ^{15}N atom percent determined from the plant tissue by mass spectrometry and the background level of the N source applied during the second year of growth (0.3664899 atom% ^{15}N, $\delta^{15}N$ = 0.51 ‰). Plant N remobilization was calculated as the ratio between plant ^{15}N content in new tissues (second year of growth) and the total plant ^{15}N content when the plants were 12 months old (end of the first year of growth).

Data analysis

All analyses were undertaken using SAS (SAS Institute Inc., Cary NC, USA, 2000). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. An analysis of variance was used to examine the main and interactive effects of nitrogen and phosphorus supply and genotype on internal cycling variables. Tukey's least significant difference test was used to distinguish among individual means where applicable, using a significance level of $P<0.05$. An analysis of covariance was used to determine if the slopes and intercepts of linear relationships between N remobilization and plant N content significantly differed among nutrient treatments or clones.

Because we were unable to directly test the contribution of roots and stems to N remobilization, we used an inferential method to determine the contribution of these woody components to N remobilization. N depletion from old foliage (y-axis) was plotted against total remobilized N (x-axis) for all plant tissues for the first half of the second year (months 12-18) and for the entire second year (months 12-24). After fitting linear equations with no intercept to the data ($y = ax$), the contribution of old foliage to total N remobilization (%) was given as the slope, a, while the contribution of woody tissues (%) was determined as $1 - a$.

Results

Treatment influences on tree growth

Growth responses in tree diameter, height (Figure 1) and mass (Figure 2) significantly increased with single-nutrient and combined N and P additions ($F_{3,30}>59$, $P<0.001$), and this response was consistent across genotypes for all harvests. Plant mass growth by the end of the experiment was

on average 5.6 times greater for the high-N high-P supply regime (798 g) (Figure 2) than for the low-N low-P supply regime (143 g). Compared to the low-N low-P supply regime, tree mass growth was increased by N additions (264 g tree^{-1}) and to a lesser extent, P additions (174 g tree^{-1}).

The main effect of clone on tree mass growth was highly significant for all the harvests (P<0.001), but relatively minor compared to the main effect of nutrient treatment, as evidenced by the *F* values (*i.e.,* 10-34 *cf.* 223-824) and the variation between extremes in plant growth, which at the experiment end ranged 5.6-fold for the nutrient treatments and 1.2-fold among genotypes (Figure 2). By the end of the experiment, plant growth in Clone B significantly exceeded that of Clone A and Clone C, but not that of Clone D, consistently for all nutrient treatments (Figure 2). The clone and nutrient treatment interaction was marginally significant for mass growth at months 12 and 18 ($F_{9,30}$> 2.3, P<0.042) but was not significant by month 24 ($F_{9,30}$ = 1.05, P=0.42).

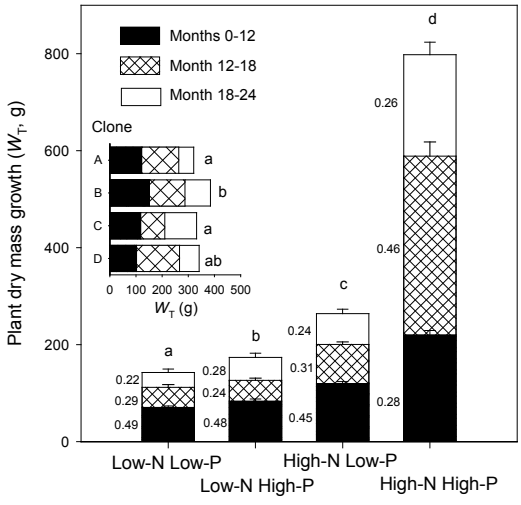

Figure 2. Comparison of plant dry mass growth across nutrient treatments and genotypes at months 12, 18 and 24. Values are presented as the means (± 1 SE) for each treatment, genotype and harvest. Different letters, among either genotypes or nutrient treatments, indicate significant differences at P≤0.05 at month 24. Numbers on the left-hand side of each vertical column of the main graph are fractions of total mass growth.

Plant growth per year varied with nutrient treatment (Figure 2). Plant mass growth during the first year was approximately 45-49% of the accumulated growth at the end of two years for all treatments except the high-N high-P treatment, for which the proportion of growth during the first year was much lower (approximately 28%). During the second year, more growth occurred in the spring and summer (months 12-18) than in the autumn and winter (months 18-24).

Diameter, height and mass of the initial cuttings at the time of planting were, as expected, not influenced by nutrient treatment ($F_{3,30}$<1.25, P>0.31), but were significantly different among clones ($F_{3,30}$> 7.6, P<0.001) such that Clone B ≥ Clone C ≥ Clone D ≥ Clone A for all measurements (data not shown). However, the analysis of covariance showed that initial plant mass was not a significant predictor of plant mass growth at months 12, 18 or 24 ($F_{1,29}$<0.23, P>0.63). This result demonstrates that initial plant mass (*i.e.,* the mass of the cuttings used in the first planting in the 4.2 L pots) did not confound the effect of nutrient treatment or genotype on plant growth at any phase of the experiment.

Changes in fascicle size and number in response to the treatments closely resembled the plant growth response (Table 1). Average mass per fascicle at month 24 scaled up with nitrogen and phosphorus supply and was 1.5 times larger (81 mg) for the high-N high-P supply regime plants than for the low-N low-P supply regime plants (55 mg). Average mass per fascicle in the unbalanced treatments (low-N high-P and high-N low-P) showed an intermediate response compared to the more extreme response of the balanced treatments (low-N low-P and high-N high-P). Similarly, the number of fascicles per plant increased with nutrient supply. The number of fascicles per plant for the high-N high-P supply regime (2322) was approximately three times the number for the low-N low-P supply regime (746), exhibiting an additive effect of mainly N, but also P, supply

on fascicle quantity. Examination of the data for the intermediate treatments indicated that it was the addition of N, not P, that stimulated fascicle production.

Fascicle size and number also tended to explain differences in growth performance among genotypes (Table 1). When compared with Clones C and D, plants from the fastest-growing Clone B had substantially larger fascicles without a significant difference in the number of fascicles per plant (Table 1), which may at least partially explain their differences in growth performance. Plants from Clone A did not fit this trend, as these plants had 1.2 - 1.8 times larger fascicles than plants from the other clones but only 0.56 - 0.62% of the total number of fascicles per plant found for the other clones. The relatively small number of total fascicles in Clone A plants may at least partially explain the poorer growth performance of plants from this clone.

Treatment influences on N remobilization

The total recovered ^{15}N ranged from 5 to 59 mg per plant. Plant ^{15}N content at the end of the first year strongly correlated and corresponded closely ($y = 0.98\,x$, r^2=0.84, P≤0.001) to plant ^{15}N content at the end of the second year. This finding was expected because ^{15}N additions were stopped during the second year. However, the correlation also provided strong evidence that plant ^{15}N was not lost from the system and that the estimates of overall remobilization in this analysis were reliable.

Total N remobilization at the end of the second experimental year was significantly greater for the high-N high-P (953 mg plant^{-1}) supply regime compared to the low-N low-P (199 mg plant^{-1}) supply regime (Table 2). The levels of nitrogen remobilization for the unbalanced treatment groups (low-N high-P and high-N low-P) were intermediate, falling between the

Table 1. Average mass per fascicle for one-year-old (old) and current-year foliage (new) and total number of fascicles per plant across nutrient treatments and clones of *Pinus radiata*.

	Mass per fascicle (mg)			Number of fascicles per plant
	Old Foliage	New Foliage		
		Month 18	Month 24	Month 24
Treatments				
Low-N Low-P	45 ± 3 a	37 ± 4 a	55 ± 6 a	746 ± 51 a
Low-N High-P	50 ± 2ab	44 ± 4 a	64 ± 7ab	715 ± 33 a
High-N Low-P	59 ± 4 b	53 ± 6ab	58 ± 5 a	1234 ± 94 b
High-N High-P	80 ± 4 c	70 ± 7 b	81 ± 8 b	2322 ± 147 c
Clones				
A	75 ± 4 c	66 ± 8 b	84 ± 7 b	858 ± 105 a
B	60 ± 4 b	53 ± 6ab	72 ± 6 b	1374 ± 202 b
C	51 ± 4ab	45 ± 4 a	56 ± 5 a	1277 ± 131 b
D	46 ± 3 a	40 ± 4 a	46 ± 5 a	1520 ± 173 b
Mean	58 ± 2	51 ± 3	64 ± 3	1255 ± 81
Analysis of variance				
T	***	***	**	***
C	***	**	***	***
C × T	ns	ns	ns	ns

Nutrient treatments were comprised of two nitrogen supply regimes (low-N=1.43 mol m^{-3} and high-N=7.14 mol m^{-3}) and two phosphorus supply regimes (low-P=0.084 mol m^{-3} and high-P=0.420 mol m^{-3}). Values are presented as the means (± 1 SE) for each treatment and clone. Significance of the main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are designated as follows: not significant (ns) (P>0.05); ** significant (P≤0.01); *** significant (P≤0.001). Separation of means was determined by a Tukey test. Different letters within treatments or clones indicate that the means were significantly different at P≤0.05.

Table 2. Plant N contents, N remobilization and N-remobilization efficiencies for all nutrient supply regimes and clones. Values are presented as the means (± 1 SE) for each treatment and clone of *Pinus radiata*.

Clone	Treatment	N content (1) at month 12 (mg plant^{-1})	N remob. (2) months 12-18 (mg plant^{-1})	N remob. (3) months 12-24 (mg plant^{-1})	N remob. efficiency months 12-18 (2) / (1)	N remob. efficiency months 12-24 (3) / (1)
A	Low-N Low-P	375 ± 36 a	146 ± 3 a	154 ± 18 a	0.40 ± 0.05 ab	0.43 ± 0.09 ab
	Low-N High-P	386 ± 31 a	208 ± 19 a	205 ± 18 ab	0.55 ± 0.04 b	0.53 ± 0.01 ab
	High-N Low-P	956 ± 55 b	199 ± 28 a	288 ± 21 b	0.20 ± 0.03 a	0.33 ± 0.02 a
	High-N High-P	1466 ± 38 c	844 ± 29 b	933 ± 45 c	0.58 ± 0.04 b	0.64 ± 0.04 b
	Mean	795 ± 96 a	350 ± 87 ab	395 ± 95 a	0.43 ± 0.05	0.48 ± 0.04 a
B	Low-N Low-P	439 ± 20 a	171 ± 20 a	208 ± 22 a	0.37 ± 0.04 ab	0.52 ± 0.08 ab
	Low-N High-P	541 ± 29 a	99 ± 30 a	239 ± 37 a	0.20 ± 0.07 a	0.44 ± 0.08 ab
	High-N Low-P	1222 ± 65 b	298 ± 31 b	453 ± 36 b	0.27 ± 0.06 ab	0.36 ± 0.02 a
	High-N High-P	1794 ± 71 c	919 ± 58 c	1064 ± 32 c	0.49 ± 0.05 b	0.63 ± 0.01 b
	Mean	999 ± 117 b	372 ± 99 b	491 ± 105 b	0.33 ± 0.04	0.49 ± 0.04 a
C	Low-N Low-P	472 ± 23 a	130 ± 3 a	220 ± 12 a	0.30 ± 0.02 a	0.44 ± 0.04 a
	Low-N High-P	552 ± 20 a	130 ± 28 a	231 ± 25 a	0.25 ± 0.07 a	0.42 ± 0.06 a
	High-N Low-P	1192 ± 53 b	370 ± 13 b	503 ± 40 b	0.31 ± 0.01 a	0.43 ± 0.04 a
	High-N High-P	1485 ± 137 b	604 ± 23 c	940 ± 16 c	0.49 ± 0.1 a	0.58 ± 0.02 a
	Mean	925 ± 96 b	308 ± 60 a	474 ± 89 b	0.34 ± 0.04	0.46 ± 0.03 a
D	Low-N Low-P	428 ± 28 a	164 ± 12 a	215 ± 15 a	0.38 ± 0.06 a	0.53 ± 0.03 a
	Low-N High-P	416 ± 26 a	161 ± 20 a	239 ± 17 a	0.41 ± 0.03 a	0.55 ± 0.03 a
	High-N Low-P	845 ± 38 b	315 ± 30 b	443 ± 15 b	0.35 ± 0.04 a	0.56 ± 0.02 a
	High-N High-P	1283 ± 79 c	830 ± 34 c	873 ± 30 c	0.62 ± 0.05 b	0.73 ± 0.01 a
	Mean	743 ± 78 a	368 ± 83 b	442 ± 80 ab	0.44 ± 0.04	0.59 ± 0.03 b
All Clones	Low-N Low-P	429 ± 15 a	153 ± 7 a	199 ± 11 a	0.36 ± 0.02 a	0.48 ± 0.03 a
	Low-N High-P	474 ± 20 a	150 ± 16 a	228 ± 12 a	0.35 ± 0.05 a	0.48 ± 0.03 a
	High-N Low-P	1054 ± 41 b	296 ± 22 b	422 ± 27 b	0.28 ± 0.03 a	0.42 ± 0.03 a
	High-N High-P	1507 ± 56 c	799 ± 39 c	953 ± 25 c	0.54 ± 0.03 b	0.65 ± 0.02 b
Overall	Mean	866 ± 49	349 ± 41	451 ± 45	0.39 ± 0.02	0.51 ± 0.02
Anova						
C		***	**	***	**	**
T		***	***	***	***	***
C × T		*	***	**	*	ns

Significance of the main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are designated as follows: not significant (ns) (P>0.05); * significant (P<0.05); ** significant (P≤0.01); *** significant (P≤0.001). Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at P≤0.05.

levels for the balanced high-N high-P and the low-N low-P supply regimes. However, the trees supplied with abundant N but not abundant P (high-N low-P) remobilized about twice the N amount (422 mg plant^{-1}) of those supplied with abundant P only (low-N high-P) (228 mg plant^{-1}).

The total N remobilized during the second year of growth was positively related to the total N content at the end of the first year of growth, and the relationship between these variables had a markedly higher intercept for the high-N high-P treatment than for the other treatments (Figure 3). Therefore, N-remobilization efficiency was significantly greater for trees that underwent the high-N high-P supply regime (0.65) compared to trees with lower N and P addition rates (with N-remobilization efficiency ranging from 0.42-0.48) (Table 2). N-remobilization efficiency was significantly greater for Clone D plants (0.59) than for all other clones (0.46-0.48), including the fast-growing Clone B (Table 2).

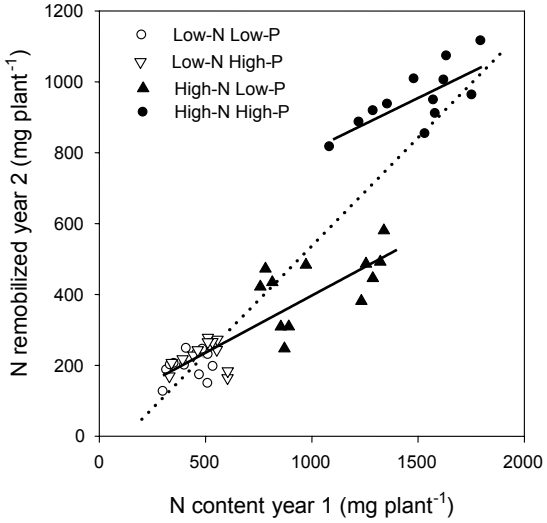

Figure 3. Relationship between total plant remobilized N during the second year of growth and total plant N content at the end of the first year of growth for the nutrient treatments: low-N low-P (open circles), low-N high-P (downward-facing open triangles), high-N low-P (upward-facing filled triangles) and high-N high-P (filled circles). For treatment high-N high-P: y = 515.7970 + 0.2923 *x*, r^2 = 0.53, P<0.01. For all other treatments: y = 74.1501 + 0.3227 *x*, r^2 = 0.74, P<0.001. Analysis of covariance analysis showed that nutrient treatment and genotype did not significantly affect the slopes and intercepts of the linear relationships between these variables. However the model clearly exhibited biases associated with nutrient treatment (y = -74.6201 + 0.6112 *x*, r^2 = 0.84, P≤0.001, dotted line) and therefore two separate models were preferred, which produced the unbiased lines shown.

Seasonal influences on N remobilization

The ratio of N remobilized during months 12-18 to total N remobilization during the experiment (Table 2) provides insight into the timing of remobilization. On average, most N was remobilized during the spring-summer season (74%) rather than the autumn-winter season (26%). However, N remobilization during spring-summer was substantially greater (84%) for the high-N high-P supply regime than for the other treatments (Table 2). Similarly, fascicle growth was concentrated mostly in spring-summer (67 - 91%) rather than autumn-winter (9 - 33%), with this trend remaining consistent across nutrient treatments and clones (Table 1).

Uptake versus remobilization

N uptake, N remobilization, and the uptake-to-remobilization ratio generally increased with N supply (Table 3). Although N uptake and N remobilization differed among the clones, the uptake-to-remobilization ratio was only influenced by the main effect of the nutrient treatments ($F_{3,30}$>14.7, P>0.001), with neither genotype ($F_{3,30}$<1.68, P>0.19) nor the nutrient treatment × genotype interaction ($F_{9,30}$<1.94, P>0.09) having a significant effect.

Remobilization sources

On average, 87% of total remobilized N came from one-year-old foliage between months 12 and 18 (Figure 4a), but this proportion was significantly smaller (64%) ($F_{1,83}$= 9.88, P=0.002) for the 12-24 month time period (Figure 4b). Neither slopes ($F_{3,39}$<2.51, P>0.07) nor intercepts ($F_{3,39}$<1.94, P>0.13) of either relationship were significantly affected by nutrient

Table 3. N uptake, N remobilization and the uptake-to-remobilization ratio across nutrient treatments of *Pinus radiata* for two periods: months 12 to 18 and months 12 to 24.

Treatment	N uptake	N remobilization	N uptake / N remobilization
	mg plant^{-1}		
Between months 12 and 18			
Low-N Low-P	183 ± 23a	153 ± 7 a	1.18 ± 0.14a
Low-N High-P	236 ± 23a	150 ± 16 a	1.73 ± 0.32ab
High-N Low-P	794 ± 110b	296 ± 22 b	2.71 ± 0.32b
High-N High-P	2060 ± 122c	799 ± 39 c	2.60 ± 0.13b
Between months 12 and 24			
Low-N Low-P	562 ± 44a	199 ± 11 a	2.82 ± 0.17a
Low-N High-P	562 ± 28a	228 ± 12 a	2.50 ± 0.14a
High-N Low-P	1661 ± 161b	422 ± 27 b	4.00 ± 0.34b
High-N High-P	3963 ± 131c	953 ± 25 c	4.18 ± 0.16b

Values are presented as the means (± 1 SE) for each treatment and remobilization period. Separation of means was determined by a Tukey test with different letters indicating significant differences at $P \leq 0.05$.

treatment or clone. These results indicate that foliage was the primary source of N remobilization to new tissues, such that old-foliage N was depleted first, then progressively replaced by N remobilization from stems and roots, which accounted for 36% of all N remobilized to new tissues at 12 to 24 months. Because most N was stored in foliage (33 - 61%) and roots (23 - 58%) with only a small proportion in stems (6 - 22%) (data not shown), it is likely that most of the 36% of total remobilized N that came from stems or roots was supplied by roots during autumn-winter of the second year.

Remobilization sinks

For all treatments, roots were the component where most of the remobilized N was used for new growth (Figure 5). N remobilization allocated to roots and foliage significantly differed among nutrient supply regimes ($F_{3,30}>4.04$, $P\leq0.016$), but were unaffected by genotypes ($F_{3,30}<2.03$, $P>0.13$) or the nutrient treatment x genotype interaction ($F_{9,30}<1.66$, $P>0.14$). Remobilization of N to foliage was significantly greater in the treatments not limited by N (high-N low-P, high-N high-P), than those limited by N (low-N low-P, low-N high-P). The fraction of N remobilized to roots in the low-N high-P treatment significantly exceeded that in the high-N low-P treatments, but no significant differences were noted between any other treatment combinations (Figure 5). Remobilization of N to stems was not significantly influenced by nutrient treatment, clone, or the interaction between these two variables.

Discussion

The absolute capacity of plants to remobilize N has been shown to increase with soil fertility for a wide variety of conifers (Carswell *et al*., 2003; Hawkins *et al*., 1999; Helmisaari, 1992). Our findings expand upon past research by showing that an imbalance in N and P supply (low-N high-P or high-N low-P) constrained N-remobilization efficiency (first hypothesis).

In this study, almost all N remobilized during the spring-summer season came from foliage (87%) and a small amount came from stems and roots (13%). However, the proportion remobilized from stems and roots increased to 36% of all remobilized N by the end of winter (second hypothesis). This is consistent with the previous research on conifers that shows foliage to be the main source of nutrients for remobilization (Millard and Proe, 1993; Nambiar and Fife, 1987). According to this

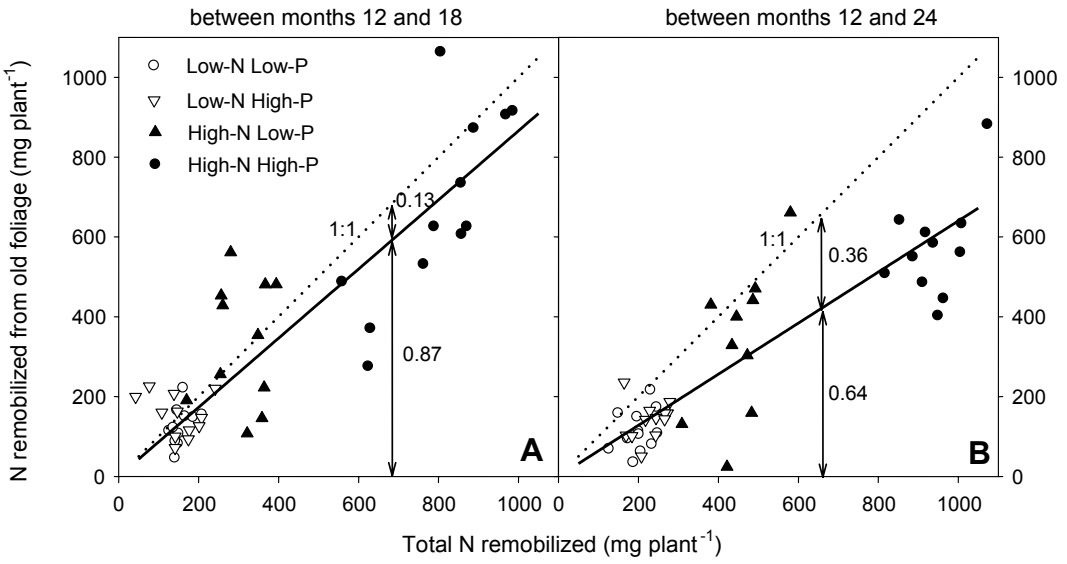

Figure 4. Comparison of remobilized (depleted) N from old foliage and total remobilized N during months (A) 12-18 and (B) 12-24. Treatments shown include: low-N low-P (open circles), low-N high-P (downward-facing open triangles), high-N low-P (upward-facing filled triangles) and high-N high-P (filled circles). For (A): $y = 0.8659\ x$, $r^2 = 0.75$, P<0.001. For (B): $y = 0.6394\ x$, $r^2 = 0.75$, P<0.001. The 1:1 lines are shown as dotted lines.

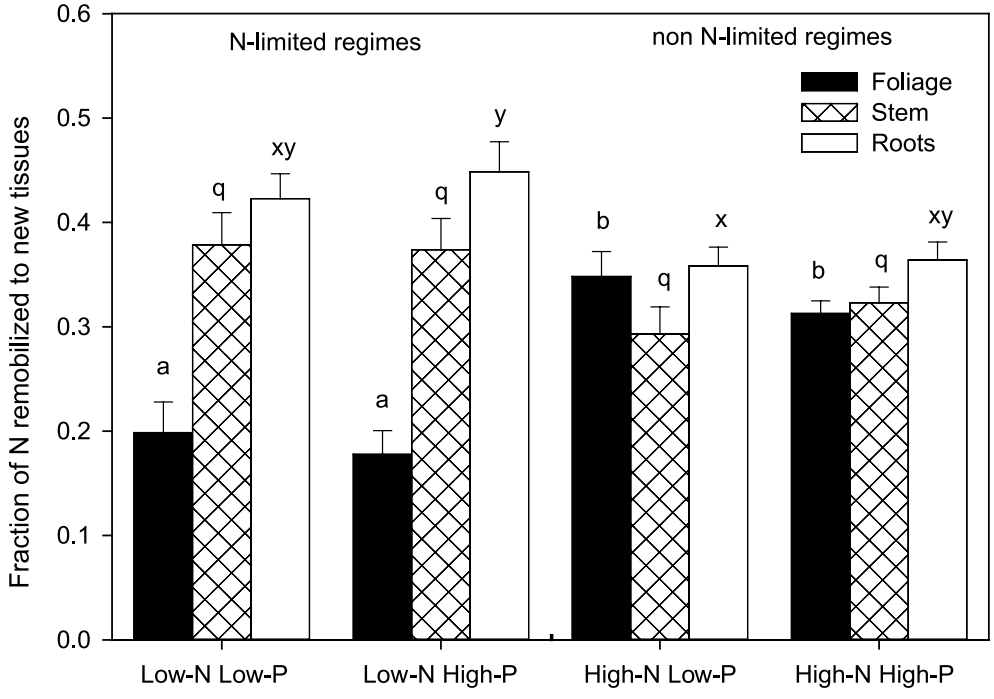

Figure 5. Fractions of N remobilized to new foliage, new stems and new roots at the end of the N remobilization experiment. Values are presented as the means (± 1 SE) for each treatment and tree component. Different letters indicate significant differences at P≤0.05 (Foliage a to b, Stems q, Roots x to y). N remobilization fractions were significantly affected by nutrient treatments, but not by clones or interactions between treatments and clones.

research, once foliage N is depleted, remobilization occurs from woody tissues (including stems as well as coarse and fine roots).

In this study there was a clear seasonal effect on N remobilization. More N remobilization occurred during spring-summer (66 - 84%) than autumn-winter (16 - 34%). Fife and Nambiar (1982) showed that in young trees of *P. radiata* in South Australia, most needle growth occurred during a period of 4 - 5 months after bud break in spring and early summer. Similarly, in this study, most needle growth occurred in spring-summer (67 - 91%), supporting the hypothesis that N remobilization is driven by sink strength (Nambiar and Fife, 1987; Nambiar and Fife, 1991). During the second half of the second year (autumn-winter), N uptake increased markedly in relation to N translocation, as has been previously observed in *Picea sitchensis* (Millard and Proe, 1993) and *Juglans nigra* × *regia* (Frak *et al*., 2005).

Millard and Proe (1993) showed that the initial growth of *Picea sitchensis* was not influenced by current N supply, but rather by N provided during the previous year. This has also been observed in the New Zealand conifer *Prumnopitys ferruginea* (Carswell *et al*., 2003). With the methods used in this study we were unable to determine if initial growth was mainly dependent on N remobilization because N uptake was also observed during the period of initial growth. In both absolute and relative terms, N remobilization was greater in the high-N high-P supply regime compared to the other treatments. Furthermore, plant uptake was greater (both in absolute and relative terms) in the high-N high-P treatment compared to the other N and P addition rates. This result suggests that processes of N remobilization and N uptake are uncoupled and independent. This finding is consistent with Nambiar (1990), who argues that shoot production and growth, rather than nutrient supply, are the key determinants of nutrient remobilization.

Forest seedlings are usually planted in winter when root regeneration and nutrient uptake are restricted by low soil temperatures (Nordborg *et al*., 2003). Under these conditions, foliage nitrogen readily remobilizes to support root growth (Nambiar and Fife, 1991). This effect has been reported to be enhanced by a nursery practice known as nutrient loading, which improves seedling growth and survival in the field (Salifu and Timmer, 2001; 2003). In this study with the young (1-2 year) plants, 44% of all N remobilized was apportioned to roots in the low-N supply regimes compared to 36% for the high-N regimes. Although the trees were not switched from one nutrient supply regime to another in this experiment, our results suggest that balanced nutrient loading of *P. radiata* in the nursery may have beneficial effects on plant growth and survival, particularly during early establishment on low-fertility soils.

Although nutrient remobilization is recognized as an important factor in the plant nutrient economy, the biochemical paths by which these nutrients are stored and remobilized are not clearly understood (Nambiar and Fife, 1991). Conroy (1992) argued that a large proportion of leaf N was associated with proteins located in the chloroplast that are mostly involved in photosynthesis, with Rubisco accounting for 25% of the total leaf N, and proteins associated with photosynthetic electron transport accounting for another 25%. Nasholm and McDonald (1990) found that the proportion of amino acids in birch tissues increased with N supply by 1 to 7%, suggesting that these amino acids might act as transport and storage compounds in addition to being the primary products of nitrogen assimilation and precursors for protein and nucleic acids. In pot trials with *Pinus sylvestris*, Warren *et al*. (2003) found that Rubisco content was in excess of the amount required for photosynthesis and that Rubisco excess was positively correlated with foliage N concentration across an N supply gradient, suggesting that Rubisco functions as a storage protein in addition to its catalytic role. Similarly, Warren and Adams (2002) found a positive correlation between foliage P and Rubisco concentrations in *Pinus*

pinaster across a P fertility gradient, suggesting that P availability controls the partitioning of N to Rubisco when P is limiting. The partitioning of foliage N to storage compounds was not measured in this study. However, N remobilization efficiency was constrained by P deficiency in the high-N low-P treatment (0.42), whereas the maximum efficiency value (0.65) was observed in the high-N high-P treatment. This finding may be the result of P deficiencies controlling the proportion of N allocated to storage proteins (Warren and Adams, 2002). Nitrogen remobilization efficiency was also constrained by N deficiency in the low-N high-P supply regime (0.48), possibly because the reduced N pool was tied up in compounds required for minimal leaf function. These results emphasize the need for a balanced nutrient supply in order for trees to realize their maximum potential for timber growth and carbon sequestration.

Nutrient remobilization efficiency has been observed to vary both among and within species. For instance, Bothwell (2001) showed that N-remobilization efficiency was higher in *Pinus contorta* (50 - 52%) than in *Picea sitchensis* (24 - 36%) on Vancouver Island (Canada). For *Pinus sylvestris,* northern European populations from colder environments exhibited higher internal nutrient cycling efficiency than southern European populations, indicating that genetics, soil and environmental factors may collectively control nutrient remobilization (Oleksyn *et al*., 2003). In this study, nitrogen remobilization did not explain differences in productivity among genotypes (third hypothesis). In fact, Clone D showed the highest N-remobilization efficiency (0.59) compared to other clones (0.48), but showed intermediate growth performance when compared with the best (Clone B) and worst (Clone A) growth performers. Therefore, other factors besides N remobilization must help to determine why certain genotypes are more productive than others.

We found that differences in growth performance may be at least partially explained by leaf area. Plants from the fastest growing Clone B had substantially larger fascicles but not significantly fewer fascicles per plant than plants from Clone C and Clone D, while plants from the slowest growing Clone A had the largest fascicles but the smallest number of fascicles per plant and leaf area. This is consistent with previous research (Cotterill and Nambiar, 1981) that suggests that differences in growth performance may be partially attributed to leaf area and phenology.

In conclusion, we examined the effects of N and P supply on N remobilization in four clones of *P. radiata*. The study showed that trees growing with an abundant and balanced nutrient supply exhibited enhanced capacity for N remobilization compared to trees exposed to N and/or P deficiencies. Spring-summer N mobilization was predominantly from old foliage, although woody tissues remobilized a substantial amount of N during autumn-winter after foliage N was depleted. Faster-growing clones did not exhibit a greater capacity for N remobilization.

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Resumen

H.E. Bown, M.S. Watt, P.W. Clinton y E.G. Mason. 2012. Influencia de los aportes de N, P y genotipo en la removilización de N en plantas de *Pinus radiata* creciendo en contenedores. Cien. Inv. Agr. 39(3): 505-520. Una alta proporción de todo el nitrógeno (N) utilizado en el crecimiento anual de la ampliamente distribuida especie forestal *Pinus radiata* proviene del N almacenado el año anterior en los tejidos de los árboles. Sin embargo, no se sabe en qué medida desbalances nutricionales, particularmente aquellos de fósforo (P), podrían cambiar la capacidad de esta especie de removilizar N. Consecuentemente, se evaluó la removilización de N en cuatro clones de *P. radiata*, sometidos a una combinación factorial de N y P, en plantas creciendo en contenedores con arena en un invernadero por dos años. Las adiciones de N fueron enriquecidas con ^{15}N al 2.5‰ (N marcado) durante el primer año. Posteriormente las plantas fueron transferidas a arena limpia, creciendo durante el segundo año con ^{15}N a niveles cercanos a la abundancia natural de N (0.3664899 porcentaje atómico ^{15}N, $\delta^{15}N$ 0.5115 ‰). Los cálculos de almacenamiento y removilización de N se basaron en la recuperación de N marcado desde los nuevos tejidos durante el segundo año de crecimiento. La removilización de N durante el segundo año fue cinco veces superior en el tratamiento de alto-N alto-P (953 mg N planta^{-1}), comparado con el de bajo-N bajo-P (199 mg N planta^{-1}), e intermedio en los tratamientos con desbalance: alto-N bajo-P (422 mg N planta^{-1}) y bajo-N alto-P (228 mg N planta^{-1}). Al expresar la removilización de N como un porcentaje del contenido de N de las plantas al final del primer año de crecimiento, se obtuvo que un 65% fue removilizado en el tratamiento de alto-N alto-P, comparado al 42-48% obtenido en los otros tratamientos al final del segundo año de crecimiento. El cuociente entre la removilización de N y la absorción de N por las raíces fue mayor en los tratamientos con bajo N comparado con los de alto N, sugiriendo que los árboles removilizan proporcionalmente más en la medida que la fertilidad declina. La mayor parte de la remobilización de N ocurrió en primavera-verano (77%), lo que coincidió con la mayor proporción de desarrollo de las acículas (80%), sugiriendo que la remobilización de N fue causada por la actividad de sus sumideros. El follaje antiguo fue la principal fuente de ciclaje interno de N, mientras que las raíces el principal sumidero. Los clones de crecimiento más rápido no mostraron una capacidad superior de remobilización, sugiriendo que el mayor desempeño en crecimiento inducido por el genotipo no es explicado por el ciclaje interno de N. En conclusión, las plantas sometidas a una oferta abundante y balanceada de nutrientes mostraron una mayor capacidad para removilizar N, comparado con aquellas plantas creciendo bajo limitantes únicas o conjuntas de N y P, y los clones que crecieron rápido no mostraron mayor capacidad para removilizar nitrógeno.

Palabras clave: Fósforo, genotipos, nitrógeno, *Pinus radiata*, removilización de nutrientes.

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