

Seasonal dynamics of nitrogen level and gas exchange in different cohorts of Scots pine needles: a conflict between nitrogen mobilization and photosynthesis?

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Abstract Needles of evergreen conifers are not only photosynthetic organs, but they may also serve as storage sites for carbohydrates and nutrients. Since nitrogen is both a component of photosynthetically active molecules and a nutrient stored in the needles and mobilized for shoot regrowth, we searched for evidence of a trade-off between needle N storage and photosynthetic capacity. Using sequential sampling, we tracked seasonal patterns in needle structure, nitrogen (N_{mass}) and carbohydrate concentration, and gas exchange in needles of all age classes (current-year, 1- and 2-year-old) present on *Pinus sylvestris* trees. In both 1- and 2-year-old needles, N_{mass} increased slightly in the spring, fell subsequently after the onset of shoot growth, followed by replenishment in 1-year-old and further decline until abscission in 2-year-old needles. However, only 2-year-old needles showed a positive correlation between N_{mass} and photosynthesis, consistent with their overall lower N level that indicated a tighter N budget. The 2-year-old needles had a higher leaf mass per area and lower photosynthesis in comparison with 1-year-old needles. They also had a lower photosynthetic nitrogen use efficiency, which suggests that in addition to N withdrawal,

structural change and biochemical modifications might have contributed to photosynthetic decline in the final year of needle life. Thus, whereas seasonal N mobilization observed in 1-year-old needles did not seem to interfere with photosynthetic potential, resorption of N could have contributed to gradual photosynthetic decline in 2-year-old needles.

Keywords *Pinus sylvestris* · Trade-off · Nitrogen storage · Carbohydrate storage · Nutrient recycling

Introduction

The demand for nutrients in perennial plants may be met through current uptake by roots, mobilization of stored reserves as well as translocation from senescing organs (Chapin et al. 1990; Millard et al. 2007). The growth of new shoots after dormancy is often supported by transfer of nutrients from storage tissues in roots, stems and branches (Millard and Grelet 2010). In evergreen species, the older leaves present on the plant during regrowth may also participate in storage (Camm 1993; Millard and Grelet 2010; Proe et al. 2000; Splittstoesser and Meyer 1971). Needles of many evergreen conifers that persist for several growing seasons present an especially interesting case in which the ontogenetic fate of an individual needle age cohort, i.e., the processes of aging and acclimation to progressive shade, is superimposed on the seasonal rhythm of resource uptake and demand by the tree, producing a dynamic, complex and only partly understood pattern of nutrient concentrations. Evergreen conifers, especially pines, with their flush-type determinate shoot growth mode, have become a favorite subject of studies on internal tree nutrient dynamics

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(Helmisaari 1992a, b; Millard et al. 2001; Nambiar and Fife 1987, 1991; Proe et al. 2000; Weikert et al. 1989).

The withdrawal of N stored in mature needles to support shoot regrowth is suggested by the beginning-of-season decline in their N concentration, as documented, for example, in *Pinus sylvestris* (Aronsson and Elowson 1980; Helmisaari 1992b), *P. densiflora* (Han et al. 2008), *P. radiata* (Nambiar and Fife 1987, 1991) and *Picea abies* (Weikert et al. 1989). In the oldest leaf cohorts, such N loss may be interpreted as recycling accompanying senescence processes. However, N withdrawal may also occur from younger needle age classes in which N levels will be fully or partly restored later in the same season, thus representing a cycle of reserve drawdown and replenishment (Aronsson and Elowson 1980; Chapin et al. 1990; Han et al. 2008; Nambiar and Fife 1987, 1991).

A large fraction of leaf N is contained in photosynthetic proteins; therefore, this nutrient is frequently used as an index of photosynthetic capacity (Evans 1989; Reich et al. 1999). Fluctuation of foliar N concentration resulting from mobilization and replenishment of this nutrient could thus contribute to changes of photosynthetic capacity at various times during the season. It is not clear, however, to what extent in various species the mobile N pool includes proteins active in photosynthesis, since other major N pools may include cell wall proteins (Harrison et al. 2009), free amino acids and other N-based compounds (Warren and Adams 2004). In *P. sylvestris*, only between 26 and 38 % of foliar N has been estimated to be allocated to photosynthetic enzymes, varying in relation to needle age and fertilization regime (Warren et al. 2003). Moreover, since amounts of Rubisco protein in conifers greatly exceed the photosynthetic requirements, it has been proposed that an inactive Rubisco form is specifically used as a storage molecule (Warren and Adams 2001; Warren et al. 2003). Accordingly, in response to heavy N fertilization, *P. sylvestris* needles accumulated Rubisco without increasing photosynthetic capacity (Millard et al. 2007). This suggests that the inactive pool of Rubisco may be used as a reserve without affecting photosynthesis. However, in *Pseudotsuga menziesii*, both photosynthetic rate and Rubisco concentration in 1-year-old needles decreased transiently during new shoot growth (Camm 1993). In *P. sylvestris* from Sweden no clear decline of photosynthetic rate in situ was observed during regrowth; however, variation of ambient temperatures might have obscured the variation in photosynthetic capacity (Troeng and Linder 1982). Thus, the effect of withdrawal of foliar N on photosynthesis is still unclear.

Scots pine (*P. sylvestris*) is a key forest species in much of Europe and Northern Asia. Temporal variability of photosynthesis in this species has been studied extensively, and several distinct features have been identified: (1) a

slow and gradual acquisition of photosynthetic capacity by young needles (Troeng and Linder 1982), (2) a late-season decline and a spring-time recovery of photosynthetic capacity in overwintering needles (Kolari et al. 2014; Mäkelä et al. 2004; Strand et al. 2002; Troeng and Linder 1982; Vogg et al. 1998) and (3) a decline in photosynthetic capacity in older age classes (Wang et al. 1995; Warren et al. 2003). However, there is still a lack of comprehensive data sets relating the variability in photosynthetic capacity in Scots pine to needle nitrogen dynamics in the different needle age classes, especially in non-juvenile, soil-grown trees. Given the dual role of needles as photosynthetic and N storage organs, it may be expected that the seasonal drawdown of N should result in a depression of photosynthetic capacity, resulting in a trade-off between these two functions.

In this study, we report on temporal dynamics of needle structure, photosynthesis, nitrogen and carbohydrate concentrations in all needle age classes of Scots pine throughout one complete growing season. By tracking changes in these key variables, we were able to test the hypotheses that: (1) production of new needles is temporally correlated with the resorption of N from older needles; (2) loss of N from needles in the intermediate age class is transient since they act as storage organs; and (3) a seasonal decrease in needle N concentration is correlated with a decreased photosynthetic capacity. Additionally, we compare the seasonal variability of N concentration to that of carbohydrates, as both represent principal storage compounds in perennial plants inhabiting seasonal environments.

Materials and methods

Study site and tree selection

Scots pine (*P. sylvestris*) stand was located in a field formerly planted with cottonwoods within the Institute of Dendrology of the Polish Academy of Sciences experimental enclosure (55°14'38"N and 17°6'6"E). Air temperature and precipitation data were obtained from a weather station located c. 400 m from the study site (see Fig. 1a). The soil was sandy loam of the Eutric Cambisol type formed on a shallow alluvial deposit. To characterize edaphic conditions of the site, in November 2015 three replicate soil cores (c. 8 cm in diameter) were obtained from below tree canopies from each 0–30 cm and 30–60 cm layers. Cores from each layer were combined into composite samples and delivered within 3 h to a certified Agricultural Laboratory for analysis. For the 0–30 cm horizon, pH (in 1 M KCl) was 5.8, and nutrient concentrations were 140 mg 100 g⁻¹ soil of total N,

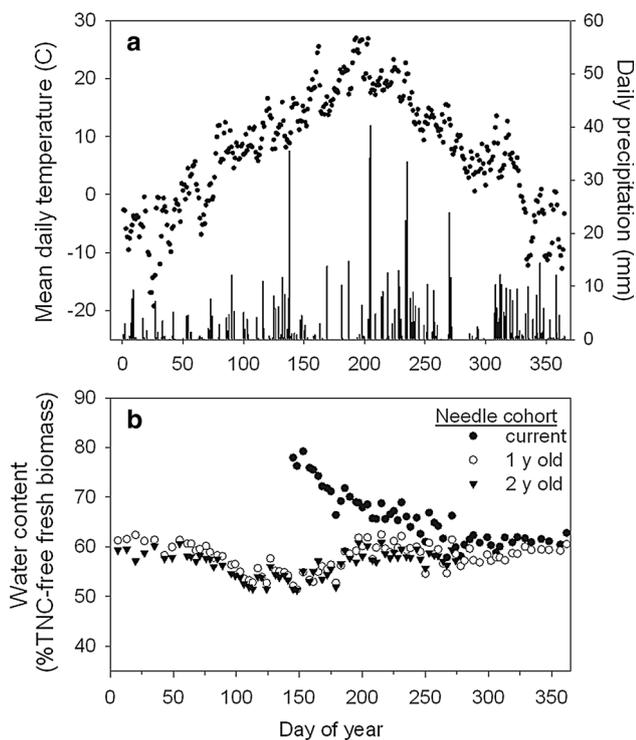


Fig. 1 Course of **a** mean daily temperature (*dots*) and precipitation (*bars*) and **b** water content of 1-year-old needles during year 2010 at the study site in Kórnik, Poland

0.54 mg 100 g⁻¹ of N-NH₄, 0.29 mg 100 g⁻¹ of N-NO₃, 10.7 mg 100 g⁻¹ of P₂O₅ and 8.8 mg 100 g⁻¹ of K₂O. For the 30–60 cm horizon, pH was 6.2, and nutrient concentrations were 84 mg 100 g⁻¹ of total N, 0.33 mg 100 g⁻¹ of N-NH₄, 0.10 mg 100 g⁻¹ of N-NO₃, 7.5 mg 100 g⁻¹ of P₂O₅ and 9.1 mg 100 g⁻¹ of K₂O.

Gas exchange

Three 15-year-old trees were selected, and a scaffolding tower was constructed to enable access to branches. In each tree, two well sunlit branches located within the third branch whorl (counting from the top), i.e., at 6.5 m above ground, were chosen and permanently marked with tape. Gas exchange measurements were conducted on 11 dates between March 26 and December 7 of the 2010 growing season using a Li-6400 analyzer equipped with the 6400-02B red-and-blue LED light source (LiCor Inc., Lincoln, Nebraska, USA). Needles from each age class present on the branch were sampled for a total of $N = 6$ gas exchange determinations per age class per date. For each measurement, three fascicles from a particular age class were removed from a branch and immediately (within 5 min after collection) separated into individual needles

and placed in a 2 × 3 cm measurement cuvette with the middle needle segments enclosed. Sticky tape was used to improve the seal of the cuvette, and a breath test was routinely used to confirm the seal. Measurements were conducted at 1200 μmol quanta m⁻² s⁻¹ to ensure saturated photosynthetic rate (based on a preliminary trial, see also Wang et al. 1995) without causing photoinhibition (Kitao et al. 2004). Leaf temperature was maintained at 22 °C (occasionally varying between 20 and 24 °C) resulting in vapor pressure deficit <1.4 kPa (usually <1 kPa). After placing needles in the cuvette, a stabilization period of several minutes was allowed for needle temperature to equilibrate and gas exchange to reach steady state after which three readings separated by at least 20 s were recorded. Measurement was typically completed within 15 min. In preliminary experiments, the detaching of needles was found not to affect their gas exchange for at least 45 min. Photosynthetic rate was calculated by inbuilt software, averaged for each sample and converted to projected needle area and dry mass bases. Because of the complex geometry of needles in the cuvette, separation of stomatal (g_s) and boundary layer conductances was not feasible; therefore, whole leaf conductance to water vapor g_{LW} (mol m⁻² s⁻¹) was calculated on the assumption that $g_{LW} = g_s$. This assumption is reasonable for narrow needles in a ventilated cuvette (Rayment et al. 2000). Internal concentration of CO₂ (C_i) was calculated following equations given in the manufacturer's handbook (LI-COR, Inc. 2005). C_i was not calculated for the final sampling date when g_{LW} was near zero.

Laboratory analyses

Needle segments used for gas exchange measurements were excised and refrigerated at 4 °C, and within a few hours, their projected areas were determined using a desktop scanner and WinSeedle software (Regent Instruments, Quebec, Canada). They were then dried at 65 °C for 72 h for the determination of dry mass. Remaining parts of the needles were also dried, and whole samples were ground in a Culatti mill (IKE Labortechnik, Staufen, Germany). Concentration of total nonstructural carbohydrates (TNC) was determined using a previously described protocol (Oleksyn et al. 2000). Nitrogen concentration was determined using an Elemental Combustion System CHNS-O 4010 (Costech Instruments, Italy/USA). To monitor tree hydraulic status, additional batches of 15–22 needles from each age class were harvested at 3- to 9-day intervals throughout the calendar year, weighed immediately after collection, dried as described above and reweighed for determination of water content in fresh biomass.

Calculations and statistics

TNC-free biomass was used as the basis for calculation of leaf mass per area index ($LMA = \text{needle dry mass} \times \text{projected area}^{-1}$; g^{-2}), nitrogen concentration (N_{mass} ; % dry mass), mass-based maximal photosynthetic rate ($A_{\text{max(mass)}}$; $\text{nmol CO}_2 \text{ g dry mass}^{-1} \text{ s}^{-1}$) and water content (% fresh mass) for each needle cohort from each tree on every sampling date. Photosynthetic nitrogen use efficiency (PNUE, $\mu\text{mol CO}_2 \text{ gN}^{-1} \text{ s}^{-1}$) was calculated as the ratio of photosynthetic rate and N concentration. The differences between needle cohorts (main effect) with respect to the various measured parameters and the effect of sampling date were evaluated with a nested repeated measures analysis of variance model. This approach, by treating trees as subjects, accounted for lack of independence between multiple measurements on the same tree. Because not all needle cohorts were present on the tree at every sampling, analyses were conducted on three data subsets: one covering the early part of the season (days 85–265) and including 1-year-old and 2-year-old needles, another covering the late part of the season (days 172–341) and including current-year and 1-year-old needles, and third, covering the middle part of the season (days 172–263) and including all three cohorts. Where the Mauchly criterion was significant, indicating that the sphericity assumption was not met, a univariate Greenhouse-Geisser adjusted test was used. Specific hypotheses associated with pairwise comparisons were evaluated using orthogonal contrasts. Arc-sin square-root transformations were used on concentration data. Additionally, relationships between N_{mass} and $A_{\text{max(mass)}}$ were explored using least-squares regression on log-transformed variables. All calculations were performed using the JMP 8.0 statistical software (SAS Institute, Cary, NC, USA).

Results

Two or three needle age classes were present on the trees on any given sampling day (i.e., current-year needles produced in spring of 2010, 1-year-old needles produced in 2009 and 2-year-old needles produced in 2008). Current-year needles emerged in May, but were sampled for the first time on day 172 (June 21) when their LMA was 70 % of season's maximum. Increase in LMA in this age class continued for the rest of the season (day 172 vs. 341, $P < 0.001$; Fig. 2a). Variability of LMA with time in both 1- and 2-year-old needles was overall significant (Supplementary Tables 1–3), with a slight early-season decline followed by an increase for the rest of the season in 1-year-old needles and until abscission in 2-year-old needles (Fig. 2a). The 2-year-old needles on sampled branches had

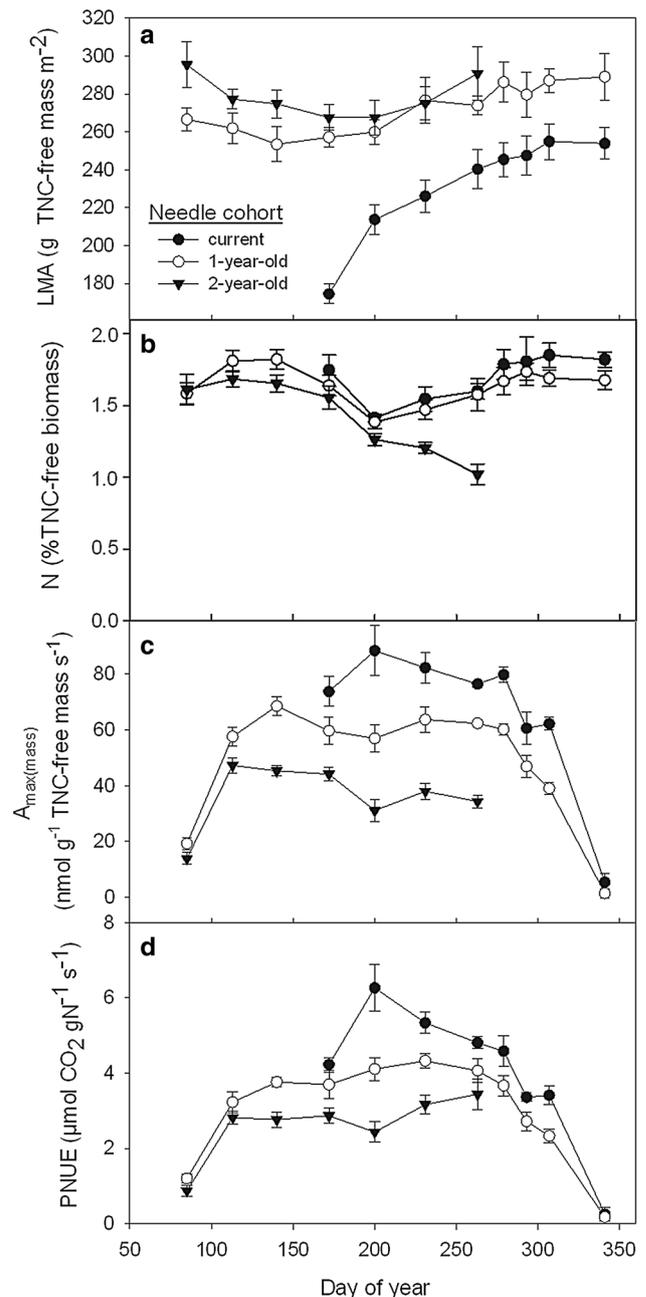


Fig. 2 Seasonal dynamics of **a** leaf mass per area (LMA), **b** needle N concentration (N_{mass}), **c** mass-based maximal photosynthetic rate ($A_{\text{max(mass)}}$) and **d** photosynthetic N use efficiency (PNUE) in current-year, 1-year-old and 2-year-old needles of *P. sylvestris* sampled throughout the 2010 growing season. Points represent daily means for a given age class, and bars are SE

all abscised in early October (by day 279). Throughout the season, LMA was consistently larger in 2-year-old needles compared to 1-year-old needles and in 1-year-old needles compared to current-year needles (cohort effect in Supplementary Tables 1–3). Needle water content varied depending on age class: In the beginning of the year, the

two preexisting age classes showed a steady level of about 60 % of fresh mass followed by a decline to 52–55 % during spring and early summer and a lasting recovery in midsummer. In current-year needles, water content was initially very high (c. 80 %) and declined to c. 60 % as needles developed (Fig. 1b).

Significant variation of N_{mass} was attributed to both time of season and needle cohort (Supplementary Tables 1–3). In 1-year-old needles, N_{mass} increased slightly at the beginning of the growing season (day 85 vs. 140, $P = 0.03$; Fig. 2b) followed by a decline during expansion of new shoots (day 140 vs. 200, $P < 0.001$) and accumulation of N after day 200 (day 200 vs. 341, $P = 0.002$). The N_{mass} in 2-year-old needles declined from day 113 (May 11) until abscission ($P < 0.001$; Fig. 2b). A transient early-summer decline of N_{mass} was apparent also in the newly formed current-year needles (day 172 vs. 200, $P = 0.02$), followed by enrichment in N later in the season ($P = 0.002$; Fig. 2b). Since LMA of these needles was still increasing, this result may indicate an early import on N into the growing needles followed by its later dilution through accumulation of biomass.

All photosynthetic parameters varied significantly with time (Supplementary Tables 1–3). $A_{\text{max}(\text{mass})}$ was very low on the first sampling day and rose to seasonal maxima that were reached on day 140 (May 20) in 1-year-old and on day 113 (April 23) in 2-year-old needles ($P < 0.001$ for both cohorts; Fig. 2c; Supplementary Table 1). Seasonal maxima were highest in current-year needles followed by 1-year-old and 2-year-old needles (cohort effect in Supplementary Table 3). Subsequently, photosynthetic capacity gradually declined for the rest of the season in 1-year-old (day 140 vs. 341, $P < 0.001$) and until abscission in 2-year-old needles (day 113 vs. 263, $P = 0.005$; Fig. 2c). A period of transient decline in $A_{\text{max}(\text{mass})}$ was also noted in these cohorts in midsummer. In current-year needles, the highest seasonal $A_{\text{max}(\text{mass})}$ was reached on day 200 (July 19), i.e., when their N_{mass} was lowest. Photosynthetic capacity of both current-year and 1-year-old needles declined to nearly zero on the last sampling day of the season, i.e., when mean daily temperature stabilized in the below-freezing range (Figs. 1, 2c). The seasonal course of area-based photosynthetic capacity $A_{\text{max}(\text{area})}$ was similar to that of $A_{\text{max}(\text{mass})}$; however, the differences in $A_{\text{max}(\text{mass})}$ between cohorts were relatively more pronounced (Figs. 2c, 3a; Supplementary Table 2), consistent with the increase in LMA with age.

Seasonal courses of PNUE mirrored those of $A_{\text{max}(\text{mass})}$; however, seasonal peaks in 1- and 2-year-old needles occurred later in the season (Fig. 2d; Supplementary Table 1). In fact, in 2-year-old needles the highest PNUE was recorded on the last sampling date before abscission. PNUE differed significantly among needle cohorts

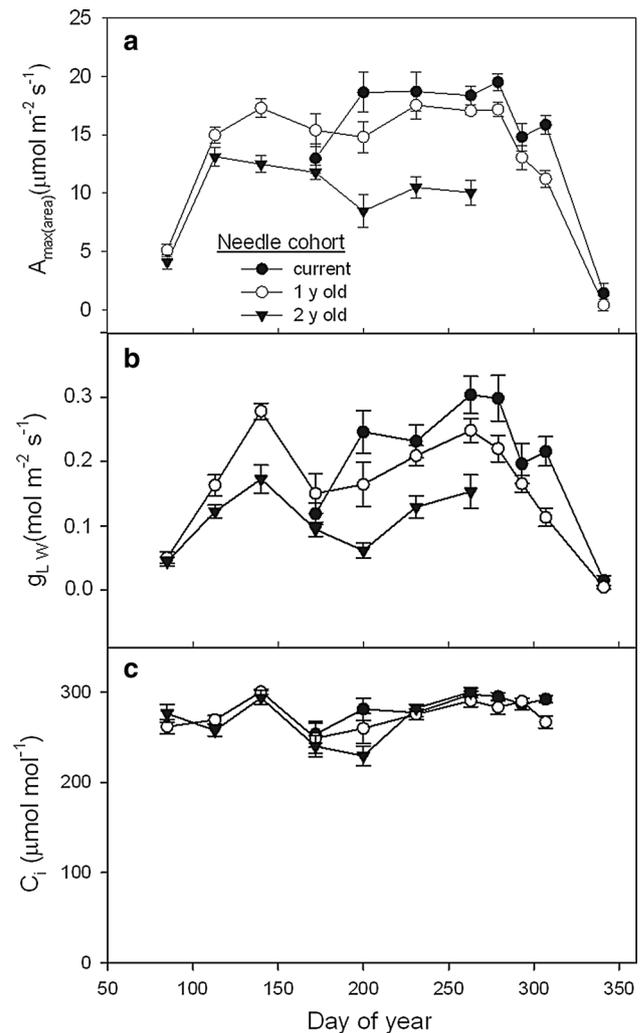


Fig. 3 Seasonal dynamics of **a** needle area-based maximal photosynthetic rate ($A_{\text{max}(\text{area})}$), **b** needle conductance to water vapor (g_{LW}) and **c** internal concentration of CO_2 (C_i) in current-year, 1-year-old and 2-year-old needles of *P. sylvestris* sampled throughout the 2010 growing season. Points represent daily means for a given age class, and bars are SE

(Supplementary Tables 1–3). Highest overall PNUE was found in current-year needles, followed by 1-year-old and 2-year-old needles. For example, on day 200 (19 July) the respective contrasts were significant at $P = 0.003$ and $P = 0.015$.

Photosynthetic rates matched g_{LW} , with the lowest $A_{\text{max}(\text{mass})}$ and $A_{\text{max}(\text{area})}$ (recorded on the earliest and latest sampling dates) corresponding to closed stomata (Figs. 2c, 3a, b). On nearly all other dates g_{LW} was consistently highest in the youngest, and lowest in the oldest, needle cohort (Fig. 3b; Supplementary Tables 1–3). In both 1-year-old and 2-year-old needles, peak g_{LW} was recorded on day 140 (May 20) followed by a transient decline in early summer (for 1-year-old needles day 140 vs. 172,

$P < 0.001$; for 2-year-old needles day 140 vs. 200, $P < 0.001$). This decline largely coincided with the period of low precipitation, lowering of needle water content and highest mean daily temperatures of the season (Fig. 1). Seasonal variability and the differences between needle cohorts with respect to C_i were less pronounced than those in photosynthesis and stomatal conductance (no significant cohort effect in Supplementary Tables 1–3), indicating a close coordination between the demand for CO_2 and stomatal opening. However, during early-summer stomatal closure, C_i dropped significantly in both 1- and 2-year-old needles ($P < 0.001$ for both), suggesting that the concurrent decline in photosynthesis could be related to stomatal limitation. Across sampling dates, $A_{\max(\text{mass})}$ (as well as $A_{\max(\text{area})}$; not shown) showed near-saturating relationships with g_{LW} in all needle age classes (Fig. 4a).

Combined across sampling dates and cohorts, $A_{\max(\text{mass})}$ showed no relationship with needle N concentration;

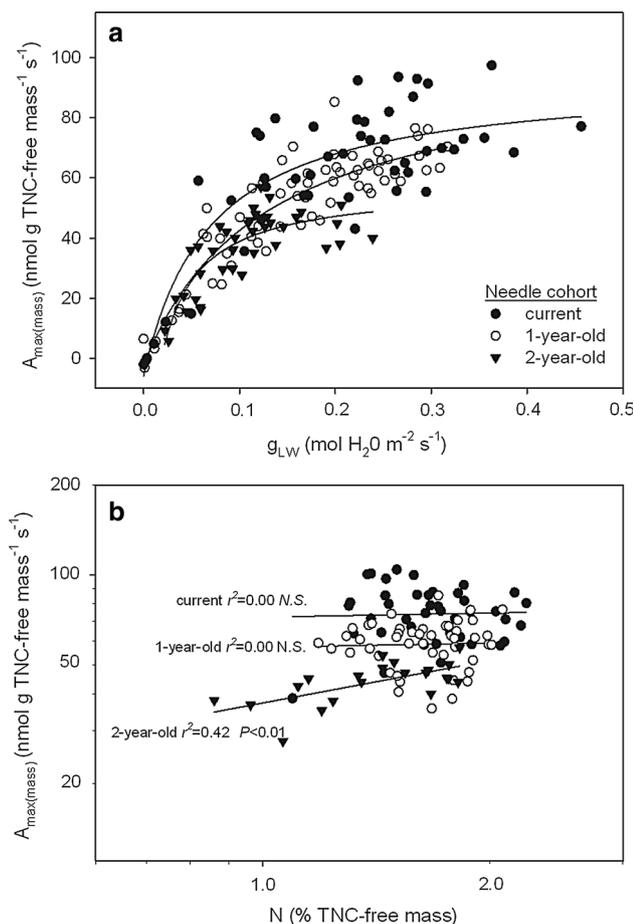


Fig. 4 Relationships between **a** mass-based maximal photosynthetic rate ($A_{\max(\text{mass})}$) and needle conductance to water vapor (g_{LW}) and **b** $A_{\max(\text{mass})}$ and needle N concentration (N_{mass}) in current-year, 1-year-old and 2-year-old needles. *Points* represent individual measurements. In **b** measurements with $g_{\text{LW}} < 0.1 \text{ mol m}^{-2} \text{ s}^{-1}$ are not included. Note log scales in **b**

however, when $A_{\max(\text{mass})}$ values corresponding to $g_{\text{LW}} < 0.1 \text{ mol m}^{-2} \text{ s}^{-1}$ were excluded (threshold chosen to exclude measurements with strong stomatal closure; Fig. 4b), the remaining values were positively correlated with N concentration (log–log transformation, $r^2 = 0.112$, $P < 0.001$, $N = 109$). Considering each cohort separately, positive correlation was confirmed for 2-year-old needles ($r^2 = 0.42$, $P = 0.002$, $N = 21$), but there was no relationship in the other cohorts (current-year needles: $r^2 = 0.00$, $P = 0.830$, $N = 40$; 1-year-old needles: $r^2 = 0.00$, $P = 0.817$, $N = 48$; Fig. 4b). When comparing needle cohorts in this subset of measurements, 2-year-old needles contained the least N and also showed the lowest $A_{\max(\text{mass})}$, with 1-year-old needles showing intermediate, and current-year needles the highest, photosynthetic capacity. The large overlap of N_{mass} ranges between needle cohorts indicated; however, that factors other than N_{mass} largely explained the decline in $A_{\max(\text{mass})}$ between years.

Seasonal courses of nonstructural carbohydrate concentration in both 1- and 2-year-old needles showed early-season peaks preceding the onset of growing season and production of new shoots followed by declines to low and steady levels later in the season ($P < 0.001$ for day 140 vs. day 200 in both cohorts; Fig. 5a; Supplementary Tables 1–3). These dynamics were largely accounted for by the fluctuation of starch (contrasts as above; Fig. 5b), whereas changes in concentration of soluble carbohydrates were less pronounced (Fig. 5c). Notably, current-year needles did not accumulate starch, but their soluble carbohydrate concentration increased with time ($P = 0.001$ for first vs. last sampling day; Fig. 5c).

Discussion

By using sequential sampling, we studied seasonal patterns in needle structure, nitrogen and carbohydrate concentration and photosynthetic rate in *P. sylvestris* needles of all age classes present on the tree. To our knowledge, this is the only data set that covers seasonal dynamics of all these variables in this species. We searched for evidence of a conflict between nitrogen mobilization from needles (i.e., their storage function) and their photosynthetic capacity. Our major finding is that the trade-off between storage and photosynthetic functions was restricted to the oldest needle cohort.

The fact that early-summer decline of N_{mass} coincided with the flush of new growth suggests that nitrogen mobilized from preexisting needles was used to support new shoot production, consistent with Hypothesis (1). This result is in line with earlier reports showing N decline during beginning-of-season regrowth in Scots pine (Aronsson and Elowson 1980; Näsholm and Ericsson 1990;

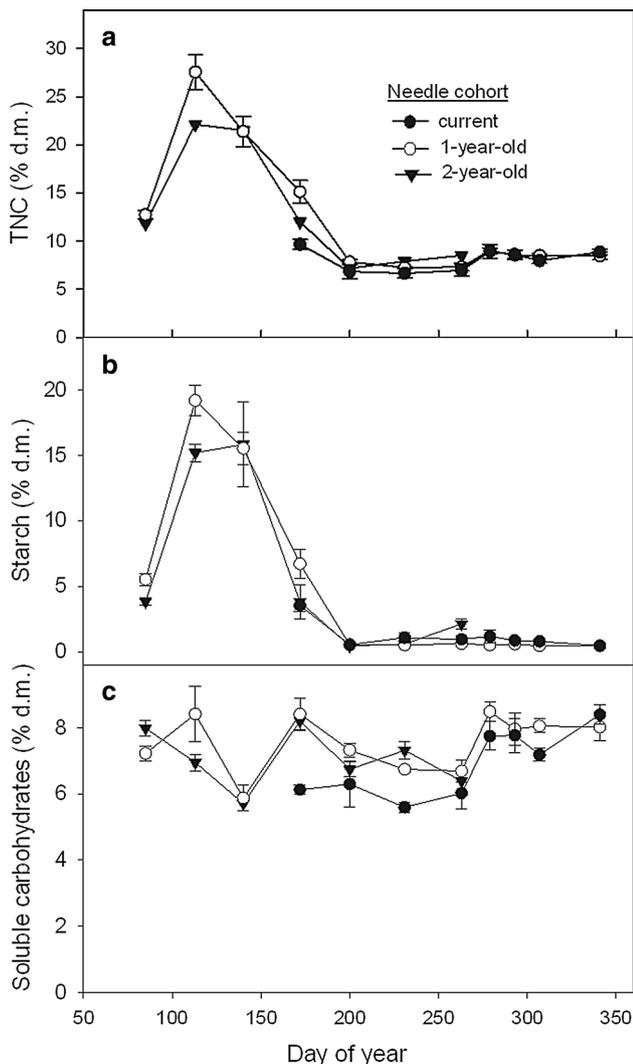


Fig. 5 Seasonal variation in carbohydrate levels in current-year, 1-year-old and 2-year-old needles of *P. sylvestris* sampled throughout the 2010 growing season: **a** total nonstructural carbohydrates (TNC), **b** starch, **c** soluble carbohydrates. Points represent daily means for a given age class, and bars are SE

Helmisaari 1992b) and other pine species (Han et al. 2008; Nambiar and Fife 1987, 1991). It is also in agreement with experimental results demonstrating that removal of some stored N by clipping of old needles inhibited production of new needles (although clipping inevitably also reduced the supply of carbohydrates; Millard et al. 2001). Mobilization of N from older conifer needles to new growth has been further confirmed by tracking of ^{15}N label (Mead and Preston 1994; Millard and Proe 1993; Proe and Millard 1994). All these results consistently indicate that N exported from needles supports shoot growth.

Both 1- and 2-year-old needles were sources of remobilized N in early summer. Between day 140 and day 200, both cohorts lost 24 % of their N contents. The subsequent recovery of N levels in 1-year-old needles is consistent

with the role of this cohort in the temporary storage of N as predicted by Hypothesis (2). Similar cycles of N mobilization/replenishment have been reported for Scots pine (Aronsson and Elowson 1980) and other conifer species (Nambiar and Fife 1991) and certainly constitute an essential adaptive mechanism of nutrient conservation in these evergreen plants. On the other hand, in 2-year-old needles N loss continued until abscission and is best interpreted as nutrient resorption accompanying senescence. This simple situation, in which preexisting needles belong to only two age classes, however, may differ from that found in many other conifers that retain needles for many more years (including Scots pine at higher latitudes). For example N fluctuation seemed to dampen after the 2nd year of needle life in *P. sylvestris* (studied at a site in Finland, where needles are retained for 4 seasons; Helmisaari 1992b) and in *P. radiata* (Nambiar and Fife 1991). Needles of different age may thus be involved in active storage processes to varying degrees, although informative, sufficiently dense time series are seldom reported. For *P. abies*, it has been suggested that the one-year-old needles serve as the primary nutrient reservoir for the new flush, whereas 2- to 3-year-old needles contribute little (Weikert et al. 1989). The principal role of previous-year conifer needles in supporting the annual growth flush has been confirmed by a recent literature review (Millard and Grelet 2010). Nevertheless, newly acquired N may be partitioned even to the oldest needle class, as shown for up to 4-year-old needles in *P. contorta* that have already remobilized much of their initial N (Mead and Preston 1994). Old needles thus not only serve as sources of N, at least in the final period of their life, but in some cases may also retain some sink potential.

Did mobilization of N from needles to support new growth affect their photosynthetic capacity? The strong inhibition of gas exchange at the beginning and end of the year was independent of N level and represented seasonal acclimation (Kolari et al. 2014; Mäkelä et al. 2004). However, during new shoot growth in spring and early summer, we found a decline in photosynthetic capacity in 1-year-old needles (by 17 % by day 200) that temporally coincided with a drop in N concentration (decline by 25 %) and was not associated with a major drop in C_i , suggesting little additional stomatal limitation. This change in photosynthesis was relatively small, suggesting the possibility for only a minor trade-off between storage and photosynthetic function in mature non-senescent needles (Camm 1993). In line with this finding, several lines of evidence indicate that the mobilizable storage N pool is not identical with the pool of photosynthetically active proteins. First, the allocation to Rubisco in 1-year-old Scots pine needles accounted for only 26–31 % of foliar N, with the balance provided by other proteins, amides and amino acids

(Warren et al. 2003). Second, the quantity of Rubisco contained in pine needles tends to be much higher than needed for photosynthesis (Warren and Adams 2001; Warren et al. 2003). Third, an application of N fertilizer may result in a many-fold increase in Rubisco concentration without an increase in photosynthesis, suggesting accumulation of an inactive storage form of Rubisco (Millard et al. 2007). Mobilization of N from needles of intermediate age classes thus may not significantly interfere with photosynthetic capacity at least under the relatively high nutritional status such as occurred in our trees.

A closer relationship between N and photosynthesis was found in 2-year-old needles, in which a withdrawal of N (loss of 25 % by day 200) was accompanied by declining photosynthetic capacity (drop by 34 % by day 200). Moreover, the gradual photosynthetic decline and N withdrawal both continued for the rest of the growing season. Thus, in the oldest needle class, in contrast to younger needles, the variability of photosynthesis throughout the growing season appeared to be related to internal tree N dynamics, in agreement with Hypothesis (3). Such slow, extended senescence, sometimes encompassing several years, is characteristic of some other evergreen species (Reich et al. 2009; Wyka and Oleksyn 2014). In some species, the N recycled during senescence originates largely from degradation of Rubisco, possibly also including the photosynthetically active pool of this enzyme (Millard and Grelet 2010). However, the fact that PNUE in 2-year-old needles of Scots pine remained stable and even increased slightly shortly before abscission suggests that there was no preferential mobilization of molecules directly involved with photosynthesis. Rather, a pool of other N-containing molecules was recycled in parallel.

Photosynthetic decline was also associated with needle aging, i.e., year-to-year transitions. The decline in $A_{\max(\text{mass})}$ in our study occurred between current-year and 1-year-old needles and between 1- and 2-year-old needles, whereas lowering of $A_{\max(\text{area})}$ occurred in 2-year-old compared to 1-year-old needles. The decline of photosynthetic capacity with age of conifer leaves appears to have been first reported by Freeland (1952) and has since been confirmed for many species of pines with different needle longevity, e.g., *Pinus aristata* (Boyce et al. 2006), *P. contorta* ssp. *latifolia* (Schoettle and Smith 1999), *P. densiflora* (Han et al. 2008), *P. heldreichii* (Oleksyn et al. 1997), *P. pinaster* (Warren 2006; Warren and Adams 2001), *P. pumila* (Kajimoto 1990), *P. sylvestris* (Escudero and Mediavilla 2003; Wang et al. 1995) as well as for other temperate and Mediterranean conifers (Chabot and Hicks 1982; Escudero and Mediavilla 2003; Kayama et al. 2002). The causes for the decline of photosynthetic capacity with age may be diverse and include: continued structural

changes leading to a dilution of N by accumulating needle biomass, an increase in CO_2 transfer conductance from atmosphere to chloroplasts (Niinemets et al. 2005), a lowering of N (Oleksyn et al. 1997 but see Escudero and Mediavilla 2003) and P concentrations (Boyce et al. 2006) or a depletion of Rubisco (Warren and Adams 2001), reallocation of N between components of photosynthetic machinery (Warren and Adams 2001) and lowering of specific Rubisco and electron transport activity (Ethier et al. 2006; Warren 2006). In our study, the lower $A_{\max(\text{mass})}$ in 1-year-old compared to current-year needles was likely predominantly caused by structural changes associated with continuing needle development, whereas when comparing 2-year-old versus 1-year-old needles, the structural changes were of much smaller magnitude. Moreover, the lowering of PNUE in each subsequent age class suggests that the photosynthetic decline is not just a result of dilution of $A_{\max(\text{mass})}$ by structural biomass, but has a physiological basis, such as an increase in resistance to CO_2 transfer or a decrease in N allocation to photosynthetically active proteins or in their activities. In 2-year-old needles, decline in $A_{\max(\text{mass})}$ could have also been caused by lower N_{mass} observed already on day 113 and related to smaller accumulation of N in the spring. Thus, 2-year-old Scots pine needles appear to lose the ability to accumulate N and become N sources. Under such low N levels, the correlation between N_{mass} and photosynthetic rate is perhaps more likely to arise than in younger, more N-rich needles. It is worth noting that conifers differ with respect to stability of their N level with needle age. While our data showed a decrease in N with age (see also Mead and Preston 1994; Oleksyn et al. 1997; Schoettle 1994), other studies have indicated stable N concentrations over consecutive years of needle life spans (Han et al. 2008; Schoettle 1994; Warren 2006). Further, with respect to age-related photosynthetic decline in conifers, structural degradation of cuticle or stomata especially during winter may cause mesophyll desiccation stress, eventually triggering senescence (Grace 1990). Such mechanism, however, is not likely to have played a role in our study since water vapor conductance markedly declined with needle age. Moreover, needle hydraulic status did not differ between 1- and 2-year-old needles. The relationships between leaf aging, N reserve mobilization, stress and photosynthesis in evergreen species clearly need to be further explored.

Our study site may be considered fertile, as suggested by the rather high needle N concentration (compared to *P. sylvestris* from other sites, e.g., Aronsson and Elowson 1980; Helmisaari 1992b; Näsholm and Ericsson 1990; Niinemets et al. 2001) and by the relatively high total soil N level and a high soil pH that is conducive to fast N mineralization. Whether the influence of internal N

mobilization on photosynthesis should be stronger at more oligotrophic sites, is debatable. On the one hand, the findings that N resorption efficiency from senescing leaves is negatively related to green leaf N concentration (Vergutz et al. 2012) and that it is reduced by fertilization (Yuan and Chen 2015) suggest that the relative degree of reserve mobilization should also be higher in N-deficient plants (Bown et al. 2012). On the other hand, mobilization of stored N is thought to be independent of current N supply and is mostly controlled by sink strength (Millard and Grelet 2010). Additional in situ monitoring and manipulative studies are needed to resolve this issue.

The sensitivity of photosynthetic rate to needle N (Smolander and Oker-Blom 1989) should depend on the extent of metabolic versus diffusive limitation of photosynthesis (Flexas et al. 2012). Drought is thus another potential factor that may modify the influence of N mobilization on photosynthesis and the within-tree nutrient cycling (Rennenberg et al. 2006). This is because: (1) photosynthesis may be limited to a greater extent by stomatal closure than by N availability as suggested by our early- to midsummer data, (2) drought alters the N uptake and transport mechanisms, and (3) drought may inhibit N mineralization and modify other biogeochemical processes affecting N availability. The control of nutrient cycling by these factors is still insufficiently understood (Rennenberg et al. 2006).

Needles in *P. sylvestris* also play a role in temporary carbohydrate storage. The annual variation of TNC is caused mostly by the variability of starch concentration that shows a peak of accumulation in the spring (in our study period, the accumulation coincided with mean daily temperatures rising to above freezing) followed by a fast depletion during shoot growth. At our time resolution, the peak of starch storage coincided with the peak of N storage, although subsequent starch depletion proceeded faster. Both age classes of preexisting needles participated in starch storage and remobilization, with a slightly smaller storage capacity of the older needles. Comparable early-season TNC peaks have been reported for *P. sylvestris* (Ericsson 1979; Oleksyn et al. 2000) and other conifers and are usually related to the phenology of shoot and root growth (Hansen and Beck 1994; Hansen et al. 1996; Kitao et al. 2004; Webb and Kilpatrick 1993). The principal difference between N and TNC storage patterns is the absence of late summer and autumn refilling of TNC reserves, possibly related to late-season activity of the root and mycorrhizal systems constituting powerful carbohydrate sinks (Hansen et al. 1996).

An important methodological issue that emerged from our study is the influence of seasonal variability of non-structural carbohydrates on the determination of needle composition and processes expressed on a needle-biomass

basis. For example, springtime nutrient levels and photosynthetic rates are “diluted” by temporary peaks of non-structural carbohydrates accumulating in needles before the onset of new shoot growth. As shown in Supplementary Fig. 1, not accounting for TNC leads not only to overestimation of LMA and occurrence of a false springtime LMA peak, but also may mask an important early-season peak of N concentration (e.g., Helmisaari 1992b; Vapaa-vuori et al. 1995; Weikert et al. 1989). This issue is not only relevant when studying temporal dynamics of needle composition and biomass-based processes but also when gathering samples from a wide geographical area where trees may be sampled at different phenological phases. Expression of results on the basis of needle area or TNC-free biomass remedies the problem.

In conclusion, tracking the dynamics of nitrogen concentration and photosynthetic capacity in Scots pine needles has supported the role of needles as sites of N storage confirming Hypotheses (1) and (2) but showed only limited evidence for a trade-off between photosynthetic capacity and N storage that operated during the final season of life of the oldest needles (Hypothesis 3). These results shed light on the consequences of internal N cycling that constitute an important aspect of resource economics of evergreen plants. Age-class specificity of the observed patterns may also be relevant to modeling of tree and stand photosynthesis (Ogée et al. 2003 but see Op de Beeck et al. 2010).

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Author contributions T.W. and J.O. designed the study, R.Ż. conducted all laboratory analyses, TW performed all photosynthetic measurements, analyzed data and wrote the manuscript, and J.O. provided consultation and editorial advice with final version of the manuscript.

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References

- Aronsson A, Elowson S (1980) Effects of irrigation and fertilization on mineral nutrients in Scots pine needles. *Ecol Bull* 32:219–228
- Bown HE, Watt MS, Clinton PW, Mason EG (2012) The influence of N and P supply and genotype on N remobilization in containerized *Pinus radiata* plants. *Cienc Investig Agrar* 39:505–520
- Boyce RL, Larson JR, Sanford RL (2006) Phosphorus and nitrogen limitations to photosynthesis in Rocky Mountain bristlecone pine (*Pinus aristata*) in Colorado. *Tree Physiol* 26:1477–1486
- Camm E (1993) Photosynthetic responses in developing and year-old Douglas fir needles during shoot development. *Trees* 8:61–66

- Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. *Annu Rev Ecol Syst* 13:229–259
- Chapin FS, Schulze E-D, Mooney HA (1990) The ecology and economics of storage in plants. *Annu Rev Ecol Syst* 21:423–447
- Ericsson A (1979) Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needle on 20-year-old Scots pine trees (*Pinus sylvestris*). *Physiol Plant* 45:270–280
- Escudero A, Mediavilla S (2003) Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of leaf lifespan. *J Ecol* 91:880–889
- Ethier G, Livingston N, Harrison D, Black T, Moran J (2006) Low stomatal and internal conductance to CO₂ versus Rubisco deactivation as determinants of the photosynthetic decline of ageing evergreen leaves. *Plant Cell Environ* 29:2168–2184
- Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78:9–19
- Flexas J, Barbour MM, Brendel O (2012) Mesophyll diffusion conductance to CO₂: an unappreciated central player in photosynthesis. *Plant Sci* 193–194:70–84
- Freeland R (1952) Effect of age of leaves upon the rate of photosynthesis in some conifers. *Plant Physiol* 27:685–690
- Grace J (1990) Cuticular water loss unlikely to explain tree-line in Scotland. *Oecologia* 84:64–68
- Han Q, Kawasaki T, Nakano T, Chiba Y (2008) Leaf-age effects on seasonal variability in photosynthetic parameters and its relationships with leaf mass per area and leaf nitrogen concentration within a *Pinus densiflora* crown. *Tree Physiol* 28:551–558
- Hansen J, Beck E (1994) Seasonal changes in the utilization and turnover of assimilation products in 8-year-old Scots pine (*Pinus sylvestris* L.) trees. *Trees* 8:172–182
- Hansen J, Vogg G, Beck E (1996) Assimilation, allocation and utilization of carbon by 3-year-old Scots pine (*Pinus sylvestris* L.) trees during winter and early spring. *Trees* 11:83–90
- Harrison MT, Edwards EJ, Farquhar GD, Nicotra AB, Evans JR (2009) Nitrogen in cell walls of sclerophyllous leaves accounts for little of the variation in photosynthetic nitrogen-use efficiency. *Plant Cell Environ* 32:259–270
- Helmisaari H-S (1992a) Nutrient retranslocation in three *Pinus sylvestris* stands. *For Ecol Manag* 51:347–367
- Helmisaari H-S (1992b) Nutrient retranslocation within the foliage of *Pinus sylvestris*. *Tree Physiol* 10:45–58
- Kajimoto T (1990) Photosynthesis and respiration of *Pinus pumila* needles in relation to needle age and season. *Ecol Res* 5:333–340
- Kayama M, Sasa K, Koike T (2002) Needle life span, photosynthetic rate and nutrient concentration of *Picea glehnii*, *P. jezoensis* and *P. abies* planted on serpentine soil in northern Japan. *Tree Physiol* 22:707–716
- Kitao M, Qu L, Koike T, Tobita H, Maruyama Y (2004) Increased susceptibility to photoinhibition in pre-existing needles experiencing low temperature at spring budbreak in Sakhalin spruce (*Picea glehnii*) seedlings. *Physiol Plant* 122:226–232
- Kolari P, Chan T, Porcar-Castell A, Bäck J, Nikinmaa E, Juurola E (2014) Field and controlled environment measurements show strong seasonal acclimation in photosynthesis and respiration potential in boreal Scots pine. *Front Plant Sci* 5:717
- Mäkelä A, Hari P, Berninger F, Hänninen H, Nikinmaa E (2004) Acclimation of photosynthetic capacity in Scots pine to the annual cycle of temperature. *Tree Physiol* 24:369–376
- Mead DJ, Preston CM (1994) Distribution and retranslocation of ¹⁵N lodgepole pine over eight growing seasons. *Tree Physiol* 14:389–402
- Millard P, Grelet G-A (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiol* 30:1083–1095
- Millard P, Proe M (1993) Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong.) Carr. as influenced by nitrogen supply. *New Phytol* 125:113–119
- Millard P, Hester A, Wendler R, Baillie G (2001) Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Funct Ecol* 15:535–543
- Millard P, Sommerkorn M, Grelet G-A (2007) Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. *New Phytol* 175:11–28
- Nambiar EKS, Fife DN (1987) Growth and nutrient retranslocation in needles of radiata pine in relation to nitrogen supply. *Ann Bot* 60:147–156
- Nambiar EKS, Fife DN (1991) Nutrient retranslocation in temperate conifers. *Tree Physiol* 9:185–207
- Näsholm T, Ericsson A (1990) Seasonal changes in amino acids, protein and total nitrogen in needles of fertilized Scots pine trees. *Tree Physiol* 6:267–281
- Niinemets Ü, Ellsworth DS, Lukjanova A, Tobias M (2001) Site fertility and the morphological and photosynthetic acclimation of *Pinus sylvestris* needles to light. *Tree Physiol* 21:1231–1244
- Niinemets Ü, Cescatti A, Rodeghiero M, Tosens T (2005) Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. *Plant Cell Environ* 28:1552–1566
- Ogée J, Brunet Y, Loustau D, Berbigier P, Delzon S (2003) MuSICA, a CO₂, water and energy multilayer, multileaf pine forest model: evaluation from hourly to yearly time scales and sensitivity analysis. *Glob Chang Biol* 9:697–717
- Oleksyn J, Tjoelker MG, Lorenc-Plucińska G, Konwińska A, Żytkowiak R, Karolewski P, Reich PB (1997) Needle CO₂ exchange, structure and defense traits in relation to needle age in *Pinus heldreichii* Christ—a relict of Tertiary flora. *Trees* 12:82–89
- Oleksyn J, Żytkowiak R, Karolewski P, Reich P, Tjoelker M (2000) Genetic and environmental control of seasonal carbohydrate dynamics in trees of diverse *Pinus sylvestris* populations. *Tree Physiol* 20:837–847
- Op de Beeck M, Gielen B, Jonckheere I, Samson R, Janssens I, Ceulemans R (2010) Needle age-related and seasonal photosynthetic capacity variation is negligible for modelling yearly gas exchange of a sparse temperate Scots pine forest. *Biogeosciences* 7:199–215
- Proe M, Millard P (1994) Relationships between nutrient supply, nitrogen partitioning and growth in young Sitka spruce (*Picea sitchensis*). *Tree Physiol* 14:75–88
- Proe MF, Midwood AJ, Craig J (2000) Use of stable isotopes to quantify nitrogen, potassium and magnesium dynamics in young Scots pine (*Pinus sylvestris*). *New Phytol* 146:461–469
- Rayment MB, Loustau D, Jarvis PG (2000) Measuring and modeling conductances of black spruce at three organizational scales: shoot, branch and canopy. *Tree Physiol* 20:713–723
- Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology* 80:1955–1969
- Reich PB, Falster DS, Ellsworth DS, Wright IJ, Westoby M, Oleksyn J, Lee TD (2009) Controls on declining carbon balance with leaf age among 10 woody species in Australian woodland: do leaves have zero daily net carbon balances when they die? *New Phytol* 183:153–166
- Rennerberg H, Loreto F, Polle A, Brillì F, Fares S, Beniwal RS, Gessler A (2006) Physiological responses of forest trees to heat and drought. *Plant Biol* 8:556–571
- Schoettle AW (1994) Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. *Tree Physiol* 14:1055–1068

- Schoettle AW, Smith WK (1999) Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* ssp. *latifolia*. *Tree Physiol* 19:13–22
- Smolander H, Oker-Blom P (1989) The effect of nitrogen content on the photosynthesis of Scots pine needles and shoots. *Ann Sci For* 46s:473s–475s
- Splittstoesser WE, Meyer MM (1971) Evergreen foliage contributions to the spring growth of *Taxus*. *Physiol Plant* 24:528–533
- Strand M, Lundmark T, Söderbergh I, Mellander P-E (2002) Impacts of seasonal air and soil temperatures on photosynthesis in Scots pine trees. *Tree Physiol* 22:839–847
- Troeng E, Linder S (1982) Gas exchange in a 20-year-old stand of Scots pine. *Physiol Plant* 54:7–14
- Vapaavuori EM, Vuorinen AH, Aphalo PJ, Smolander H (1995) Relationship between net photosynthesis and nitrogen in Scots pine: seasonal variation in seedlings and shoots. *Plant Soil* 168–169:263–270
- Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecol Monogr* 82:205–220
- Vogg G, Heim R, Hansen J, Schäfer C, Beck E (1998) Frost hardening and photosynthetic performance of Scots pine (*Pinus sylvestris* L.) needles. I. Seasonal changes in the photosynthetic apparatus and its function. *Planta* 204:193–200
- Wang K, Kellomäki S, Laitinen K (1995) Effects of needle age, long-term temperature and CO₂ treatments on the photosynthesis of Scots pine. *Tree Physiol* 15:211–218
- Warren C (2006) Why does photosynthesis decrease with needle age in *Pinus pinaster*? *Trees* 20:157–164
- Warren C, Adams M (2001) Distribution of N, Rubisco and photosynthesis in *Pinus pinaster* and acclimation to light. *Plant Cell Environ* 24:597–609
- Warren CR, Adams MA (2004) Evergreen trees do not maximize instantaneous photosynthesis. *Trends Plant Sci* 9:270–274
- Warren C, Dreyer E, Adams M (2003) Photosynthesis-Rubisco relationships in foliage of *Pinus sylvestris* in response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores. *Trees* 17:359–366
- Webb WL, Kilpatrick KJ (1993) Starch content in Douglas-fir: diurnal and seasonal dynamics. *For Sci* 39:359–367
- Weikert RM, Wedler M, Lippert M, Schramel P, Lange OL (1989) Photosynthetic performance, chloroplast pigments, and mineral content of various needle age classes of spruce (*Picea abies*) with and without the new flush: an experimental approach for analysing forest decline phenomena. *Trees* 3:161–172
- Wyka TP, Oleksyn J (2014) Photosynthetic ecophysiology of evergreen leaves in the woody angiosperms—a review. *Dendrobiology* 72:3–27
- Yuan ZY, Chen HYH (2015) Negative effects of fertilization on plant nutrient resorption. *Ecology* 96:373–380