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Shading effects needle xylem traits and leaf gas exchange parameters in Scots pine †

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Abstract: Forest productivity is closely related to how effectively woody plants utilize the most important environmental factors—light and moisture. Assessment of ecological plasticity of structural and functional traits in woody plants is necessary to predict the dynamics of forest communities in the changing natural environment and climate. In this study, needle xylem anatomical and hydraulic traits and their relationships with leaf CO_2/H_2O-gas exchange parameters were investigated in Scots pine (*Pinus sylvestris* L.) trees during natural reforestation after clear-cutting of boreal pine forest in Eastern Fennoscandia. We analyzed the effect of shading on needle structural and functional traits in Scots pine trees of the same age in a clear-cut site and under bilberry-type pine forest canopy in the middle taiga. The highest values of tracheid lumen diameter (D_{95}), number of tracheids per needle (T_{num}) and xylem area per needle (A_x), theoretical needle hydraulic conductivity (K_{th_n}) and theoretical leaf-specific hydraulic conductivity(K_{s_leaf}), stomatal conductance (g_s), rates of photosynthesis (A) and transpiration (E), number of stomata per unit needle area (N_{st}) and, on the contrary, the lowest values of photosynthetic water use efficiency (WUE_i, WUE) and plasticity index (PI) of all structural and functional traits were noted in Scots pine trees growing in the clear-cut and getting sufficient amounts of light. At the same time, the values of theoretical needle xylem-specific hydraulic conductivity (K_{s_xylem}) were similar in habitats with high (clear-cut site), medium (shading in the clear-cut), and low (forest canopy) light levels. The features of the relationship between the hydraulic structure, photosynthetic capacity, and water use efficiency in Scots pine trees under different habitat conditions are discussed.

Keywords: coniferous plants; needle xylem traits; hydraulic conductivity; photosynthesis; transpiration; environmental factors

1. Introduction

Forest productivity is closely related to how effectively woody plants utilize the most important environmental factors – light and moisture. The photosynthetic capacity, implemented by the assimilating surface (foliage), and competition for resources (light, water, nutrients) constrain the growth of trees. The xylem, which is the main water conducting tissue in terrestrial plants, supplies water and nutrients to the plant's photosynthetic and growing tissues, thus linking together the water and the carbon cycles [1,2]. The efficiency and safety of xylem functioning largely determine the growth, productivity and survival of plants in changing environmental conditions [3–5]. At the same time, there exist functional relationships between photosynthetic activity, water use efficiency, and leaf structure, which reflect the physiological traits and ecological strategies of species [6–8]. Therefore, assessment of the ecological plasticity of structural and functional traits in woody plants is of special value for predicting the dynamics of forest communities in the changing natural environment and climate. This study aimed to estimate the response of needle xylem anatomical and hydraulic traits and their relationships with CO_2/H_2O-gas exchange parameters in Scots pine (*Pinus sylvestris* L.) trees during natural reforestation after clear-cutting of a bilberry-type pine forest in Eastern Fennoscandia (Southern Karelia). We analyzed intraspecific variability of needle structural and functional characteristics in Scots pine trees of the same age growing in shade in a clear-cut site and under the canopy of boreal pine forest relative to full sunlight in the clear-cut.

2. Materials and Methods

2.1. Study Area and Vegetation

This study was carried out in the European part of the middle taiga (southern Karelia) at adjacent sample plots (SP) situated in an 8-year-old clear-cut Scots pine stand and a mature 95-year-old bilberry-type pine stand in July 2017. The climate in the study area is of the subarctic type [9], characterized by a relatively evenly distribution of precipitation over a year (550–750 mm annual mean). Mean monthly air temperatures in January and July are –11 °C and +16 °C, respectively. Total incoming solar radiation over the growing season does not exceed 1130 MJ m^{-2}. The trees selected for measurements were Scots pine (*Pinus sylvestris* L.) regrowth trees from the same age group (8–10 years old) growing in the clear-cut site (SP_{HL} with high light level, 100%), and under shade in the clear-cut (SP_{ML} with medium light level, 60%) and under pine forest canopy (SP_{LL} with low light level, 20%). Five Scots pine trees were sampled in each SP.

2.2. Needle Xylem Traits

To determine xylem anatomical and hydraulic traits we sampled 1-year-old needles from 5 model trees in each SP in the last third of July 2017. The samples were fixed in situ in 3% glutaraldehyde solution and placed in the cold. The leaf anatomy was studied from serial sections using the classical paraffin technique and subsequent safranin staining. Thin cross-sections were cut from each sample using Frigomobil 1205 freezing microtome (Reichert–Jung, Heidelberg, Germany). The sections (n = 375) were examined under an AxioImager A1 light microscope (Carl Zeiss, Germany) at ×10 magnification. Images were recorded using an ADFPRO03 camera (ADF Optics, China) and ADF Image Capture software (ADF Optics, China). Digital images were processed with ImageJ v. 1.50 (NIH, USA) to measure radial diameters of the lumen of xylem tracheids, xylem area per needle (Ax, μm^2), and to count the number of stomata per unit needle area ($N_{st,}$ N mm^{-2}) and the number of tracheids per needle (T_{num}). The mean diameter (D_{95}, μm) was calculated for this subset of tracheids. The tracheid theoretical hydraulic conductivity (K_{th_t}, kg m s^{-1} MPa^{-1}) was calculated according to the Hagen–Poiseuille law [10]. The needle theoretical hydraulic conductivity (K_{th_n}, kg m s^{-1} MPa^{-1}) was calculated as the sum of all K_{th_t} per needle. Then, the xylem-specific hydraulic conductivity (K_{s_xylem}, kg m^{-1} s^{-1} MPa^{-1}) and needle-specific hydraulic conductivity (K_{s_leaf}, kg m^{-1} s^{-1} MPa^{-1}) were calculated from the K_{th_n} /A_x and K_{th_n}/A_{needle} ratios, respectively [11]. The plasticity index (PI) was calculated from the (X_{max} – X_{min})/X_{max} ratio, where X_{max} and X_{min} represent the mean maximum and minimum parameter, respectively [12].

2.3. Leaf Gas Exchange Parameters

All gas exchange measurements were performed using the portable photosynthesis system LI-6400XT (LI-COR Inc., Lincoln, NE, USA) fitted with a CO_2 mixer, the standard 2 cm × 3 cm leaf chamber and a light source LI-6400-02B LED (LI-COR Inc., Lincoln, NE, USA) in July 2017. Field measurements of stomatal conductance (g_s, mol m^{-2} s^{-1}), photosynthesis (A, μmol m^{-2} s^{-1}) and transpiration (E, mmol m^{-2} s^{-1}) in the needles of trees at each SP were conducted between 10 a.m. and 4 p.m. Throughout the measurements, the reference CO_2 concentration was maintained at 400 μmol mol^{-1}, light intensity was set to 1600 μmol m^{-2} s^{-1}, air flow rate was 400 μmol s^{-1}, while chamber temperature was kept at 25 °C. The relative air humidity was kept between 50% and 70% during the measurements. The instantaneous photosynthetic water use efficiency (*WUE*, μmol CO_2 mmol^{-1} H_2O) and intrinsic WUE_i were calculated from the A/E and A/g_s ratios, respectively. The data were analyzed with Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

Our previous study [13] revealed significant differences between the habitat conditions of SPs. Microclimate in the clear-cut SP_{HL} in July featured higher mean daytime values of photosynthetically active radiation (1218 μmol m^{-2} s^{-1}), water vapour pressure deficit (2.2 kPa), air (27.1 °C) and soil (16.1 °C) temperatures compared to the forest SP_{LL} (240 μmol m^{-2} s^{-1}, 1.3 kPa, 22.4 °C and 13.8 °C, respectively). The contrasting habitat conditions in the clear-cut SP_{HL} versus the shaded conditions in the clear-cut SP_{ML} and the forest SP_{LL} have significantly influenced the needle xylem traits and leaf gas exchange parameters in all trees (Figure 1), except theoretical needle xylem-specific hydraulic conductivity (K_{s_xylem}), whose values were relatively stable across the SPs (0.75–0.80 kg m^{-1} s^{-1} MPa^{-1}) compared to other characteristics.

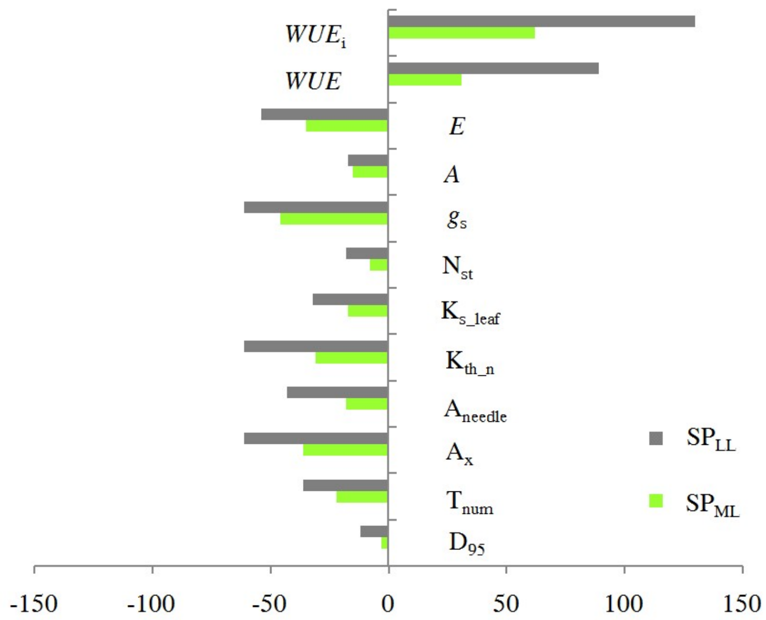

Figure 1. Variability (%) of needle xylem traits and leaf gas exchange parameters under shaded conditions in the clear-cut SP_{ML} and the forest SP_{LL} relative to full sunlight in the clear-cut SP_{HL}.

The highest values of tracheid lumen diameter (D_{95}), number of tracheids per needle (T_{num}) and xylem area per needle (A_x), needle area (A_{needle}), theoretical needle hydraulic conductivity (K_{th_n}) and theoretical leaf-specific hydraulic conductivity(K_{s_leaf}), number of stomata per unit needle area (N_{st}), stomatal conductance (g_s), rates of photosynthesis (A) and transpiration (E) and, on the contrary, the lowest values of photosynthetic water use efficiency (WUE_i, *WUE*) were noted in Scots pine trees growing in the clear-cut SP_{HL} and getting sufficient amounts of light. At the same time, the values of theoretical needle xylem-specific hydraulic conductivity (K_{s_xylem}) were similar ($p > 0.05$) in habitats with high (clear-cut site), medium (shading in the clear-cut), and low (forest canopy) light levels (Table 1).

Table 1. *p* values from the Tukey's honest significant difference (HSD) test showing the probability of differences in the needle xylem traits and leaf gas exchange parameters between the clear-cut SP_{HL} and shading in the clear-cut SP_{ML} or the forest SP_{LL} and between SP_{ML} and SP_{LL}.

Parameter	P value		
	$SP_{HL} \times SP_{ML}$	$SP_{HL} \times SP_{LL}$	$SP_{ML} \times SP_{LL}$
D_{95}	*	***	***
T_{num}	***	***	***
A_x	***	***	***
A_{needle}	***	***	***
K_{th_n}	***	***	***
K_{s_leaf}	**	***	*
K_{s_xylem}	***ns***	***ns***	***ns***
N_{st}	***	***	***
g_s	***	***	***
A	***	***	***ns***
E	***	***	***
WUE	***	***	***
WUE_i	***	***	***

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *ns* – not significant ($p > 0.05$).

Variation of xylem anatomical characteristics in woody plants is known to reflect the processes of cells division in the cambial zone, radial cell enlargement and cell wall formation, which are under hormonal and genetic control and influenced directly and indirectly by external environmental conditions [3]. Augmentation of needle xylem anatomical traits in trees growing in the high-light conditions of the clear-cut SP_{HL} was apparently due to a general intensification of growth processes. In turn, the increase of theoretical needle hydraulic conductivity (K_{th_n}) and theoretical leaf-specific hydraulic conductivity (K_{s_leaf}) in trees from the clear-cut SP_{HL} is supposed to promote water supply to leaves and the processes of photosynthesis and transpiration [14, 15], in agreement with our data on the highest values of stomatal conductance and rates of photosynthesis and transpiration in trees at the clear-cut SP_{HL}. The rise of photosynthetic water use efficiency (*WUE*i, *WUE*) in Scots pine trees at the forest SP_{LL} is probably due to stomatal restriction of transpiration losses relative to the reduction of the CO_2 assimilation rate (Figure 1) and, hence, a higher water use savings compared to the clear-cut SP_{HL}. Also, the greatest reduction in both K_{th_n} and K_{s_leaf} observed in regrowth trees at the forest SP_{LL} is likely a result of the limited availability of soil moisture due to higher competition for water resources in a mature tree stand, caused in particular by moisture interception by roots of adult trees [13]. The decrease of both *WUE*i and *WUE* in Scots pine in the clear-cut SP_{HL} may have the aim of enhancing the water supply to compensate for the transpiration losses under high light conditions as compared to the moderate shade in the clear-cut SP_{ML} and the forest SP_{LL}. At the same time, stability of the theoretical needle xylem-specific hydraulic conductivity (K_{s_xylem}) reflects the intraspecific similarity of the internal properties of Scots pine xylem tissues in different habitat conditions (Table 1).

On the other hand, the lowest values of the plasticity index (PI) of almost all needle xylem traits and leaf gas exchange parameters were noted in trees exposed to full sunlight in the clear-cut SP_{HL} (Figure 2). This fact may be related to the lowest variability (30%) of daytime irradiance in the clear-cut SP_{HL} and, conversely, its greatest (70%) variability under the forest canopy in SP_{LL} due to sun flecks and glare. Importantly, the lowest (0.23–0.36) and the highest (0.73–0.89) PI values were found, respectively, for needle anatomic traits (D_{95}, A_{needle}) and hydraulic traits (K_{th_n}, K_{s_xylem}, K_{s_leaf}) in all the habitats.

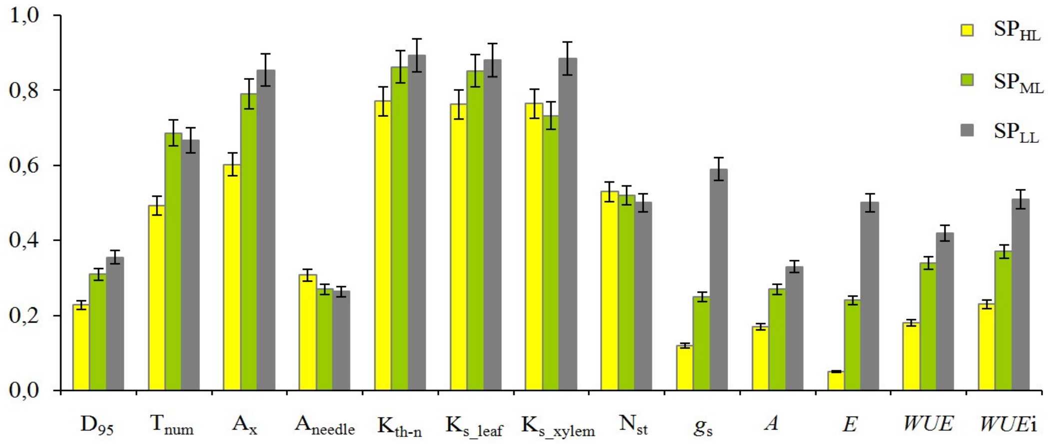

Figure 2. Plasticity index (PI) of needle xylem traits and leaf gas exchange parameters in the clear-cut SP_{HL}, in the shaded clear-cut SP_{ML}, and in the forest SP_{LL}.

4. Conclusions

The study revealed significant variability of needle xylem anatomical and hydraulic traits and CO_2/H_2O gas exchange parameters of Scots pine trees growing in the different habitats. However, theoretical needle xylem-specific hydraulic conductivity (K_{s_xylem}) proved to be quite stable across the habitats, reflecting intraspecific similarity of xylem tissue intrinsic properties in Scots pine trees growing in different SPs. The observed stability of the hydraulic capacity of needle xylem across different habitats may be part of a conservative resource use strategy, which seems to be adaptive for woody plants, in particular boreal conifers. The estimations of intraspecific features of hydraulic structure coordination, photosynthetic capacity and water use efficiency can be useful for revealing the mechanisms underlying the stability of forest ecosystems and restoration of their structure and species composition in a changing natural environment and climate.

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Conflicts of Interest: The authors declare no conflict of interest.

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