

Leaf- and ecosystem-scale photosynthetic parameters for the overstory and understory of boreal forests in interior Alaska

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Abstract

Photosynthetic parameters are key for predicting the carbon cycle and fluxes in terrestrial ecosystems. In northern high-latitude regions where cold temperatures limit available nitrogen for plants, the photosynthetic parameters are tightly linked with the nitrogen content. Here, we present the leaf- and ecosystem-scale photosynthetic parameters, the maximum carboxylation rate ($v_{C\max 25}$), based on leaf chamber measurements and eddy covariance data from two mature black spruce forests and one young aspen-birch forest in interior Alaska. The leaf carbon to nitrogen ratio (C:N ratio) explained most of the variations in $v_{C\max 25}$ across the five vascular plants (*Picea mariana*, *Betula glandulosa*, *Ledum groenlandicum*, *Betula papyrifera*, and *Populus tremuloides*) ($R^2 = 0.68$; $p < 0.01$), including the overstory and understory, from the three forests. Each plant had a small within-species variability of $v_{C\max 25}$ and leaf C:N, suggesting that plants have a specific potential for available N in photosynthesis. This result indicates that the canopy structure and community composition are presumably important factors for modeling the canopy photosynthesis across boreal forests in interior Alaska. The ecosystem-scale $v_{C\max 25}$ determined from the eddy covariance data further supported this hypothesis.

Key words: Alaska, Boreal forest, Ecophysiology, Leaf C:N ratio, Photosynthesis

1. Introduction

Coupling carbon and nitrogen cycles is important for predicting greenhouse gas budgets of high-latitude ecosystems (Euskirchen *et al.*, 2010) where a substantial warming trend has been observed (Chapin *et al.*, 2005). In high-latitude ecosystems, cold temperatures limit decomposition and the available nitrogen (Euskirchen *et al.*, 2010). Warming thus increases microbial activity and available nitrogen, potentially inducing the stimulation of photosynthetic capacities (Euskirchen *et al.*, 2010; Ueyama *et al.*, 2009). These processes, increases in decomposition and photosynthesis, could change the carbon budget in high-latitude ecosystems (Clein *et al.*, 2002; Euskirchen *et al.*, 2010).

Understanding the photosynthetic parameters of high-latitude ecosystems is important for predicting the high-latitude carbon cycle (Clein *et al.*, 2002). Photosynthetic parameters in the North American boreal forest were reported mostly at its southern edge in Canada (e.g., Dang *et al.*, 1997, 1998), but are rarely reported for its northern edge in interior Alaska. Black spruce (*Picea mariana*) is the major overstory species of the North American

boreal forest (Girardin *et al.*, 2016). In addition to the overstory, understory and pioneer species after forest fires are also important components of North American boreal forests (Ikawa *et al.*, 2015; Welp *et al.*, 2007).

The maximum carboxylation rate, an index of photosynthetic capacity under light saturation under current atmospheric CO₂ concentration (Farquhar *et al.*, 1980), is a key parameter for predicting the carbon cycle in terrestrial ecosystems (Bonan *et al.*, 2011). Variations in the carboxylation rate within a forest (Dang *et al.*, 1997) and between forests (Bonan *et al.*, 2012) characterize forest productivities, and are thus necessary information for ecosystem modeling (Bonan *et al.*, 2011, 2012). As the maximum carboxylation rate is generally related to leaf nitrogen content (Kattge *et al.*, 2009), this parameter links the carbon and nitrogen cycles (Clein *et al.*, 2002).

In this study, we evaluated the photosynthetic parameters for overstory and understory plants of two black spruce and one young aspen-birch forests in interior Alaska, based on leaf chamber measurements for the leaf scale and an inversion of a canopy photosynthesis model using eddy covariance fluxes (Ueyama *et al.*, 2016) for the ecosystem scale. We show a single relationship between the maximum carboxylation rates and the leaf carbon to nitrogen ratios (C:N ratio) within and between the forests, and discuss the linkage of the leaf- and ecosystem-scale photosynthetic parameters.

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2. Measurements

2.1 Study sites

The measurements were conducted in two mature black spruce (*Picea mariana*) forests, and in one aspen-birch forest 12-years after a forest fire in 2004 in interior Alaska. The three sites experienced a similar boreal climate. One mature forest was located on the campus of the University of Alaska Fairbanks (FAI, 64°52'N, 147°51'W, elevation 155 m; Iwata *et al.*, 2012; Ueyama *et al.*, 2014a) and the other mature forest was located near Delta Junction (DLS, 63°54'N, 145°40'W, elevation 492 m; Randerson *et al.*, 2006; Welp *et al.*, 2007). The aspen-birch forest was located in the Poker Flat Research Range (PFF, 65°08'N, 147°26'W, elevation 491 m; Iwata *et al.*, 2011).

The dominant overstory of the FAI and DLS forests was black spruce; FAI consisted of an open canopy on permafrost, whereas DLS consisted of a closed canopy on a non-permafrost soil. The forest floor at FAI was completely covered by *Sphagnum* and feather mosses and understory shrubs (e.g., *Betula glandulosa*, *Ledum groenlandicum*, *Vaccinium uliginosum*, *Vaccinium vitis-idaea*, *Larix laricina*, and *Rubus chamaemorus*). The forest floor at DLS was covered by feather mosses (*Hylocomium splendens*, and *Pleurozium schreberi*), lichens (*Cladonia spp.*, and *Stereocaulon spp.*), and understory species (e.g., *Ledum palustre*, *Vaccinium uliginosum*, and *Vaccinium vitis-idaea*). The ages of black spruce trees were 36 to 119 years (mean of 86 years) for FAI, and 74 to 132 years (mean of 103 years) for DLS in 2012 (Ueyama *et al.*, 2014b). The approximate mean canopy heights were 3 m for FAI, and 4 m for DLS.

The aspen-birch forest regrew at a fire scar burned in 2004 (PFF; Iwata *et al.*, 2011). The overstory of PFF was paper birch (*Betula papyrifera*) and trembling aspen (*Populus tremuloides*), and understory vegetation (e.g., *Picea mariana*, *Vaccinium uliginosum*, *Salix spp.*, *Betula glandulosa*, *Carex lasiocarpa*, and *Polytrichum commune*) was abundant in 2016. The mean and standard deviation of tree heights were 2.9 ± 0.8 m for *Betula papyrifera* ($n = 75$), 1.5 ± 0.8 m for *Populus tremuloides*

($n = 59$), and 0.24 ± 0.19 m for *Picea mariana* ($n = 152$) in August 2016.

2.2 Leaf chamber system

We developed an open chamber system for measuring the photosynthetic and transpiration rates. The branch chamber was made by a 1.5-liter transparent plastic cylindrical container (B-2265, Iwasaki Industry Inc., Japan). Air was sent to the chamber using a pump (MV-10, Enomoto Micro Pump Manufacturing Co., Japan) with a 62-liter buffer. The flow rate was recorded using a mass flow sensor (Model 3810 S, Kofloc, Japan), and ranged between 1.9 and 2.3 liters minute $^{-1}$. Air within the chamber was mixed with two fans for eliminating the leaf boundary resistance. CO₂ concentrations inside and outside the chamber were measured using an infrared gas analyzer (GMP343, Vaisala, Finland). To measure CO₂ concentrations, flow lines were switched using solenoid valves (USG3-6-2-E, CKD, Japan). To remove dust, an air filter (RAWP 1.2 μm, Merck Millipore Ltd., USA) was placed just before the gas analyzer. The air temperature and relative humidity were measured inside and outside the chamber using temperature and humidity sensors (HMP60, Vaisala, Finland) with a ventilated shield. Leaf temperature was measured using a thermocouple thermometer. Photosynthetically photon flux density (PPFD) surrounding the chamber was measured using a quantum sensor (LI-190, Li-Cor, USA), where the quantum sensor was installed in another of the same transparent plastic container. The measurements were controlled by a logger (CR1000, Campbell Scientific Inc., USA), and data were recorded every second. The dilution effect due to water vapor fluctuations was corrected before calculating the photosynthetic rate (Harazono *et al.*, 2015).

2.3 Field measurements

Leaf photosynthetic and transpiration rates were measured between 10:00 and 16:00 Alaska daylight time from late July to early August in 2016 using the open chamber system at the three sites (Table 1). Measurements were taken for sunlit leaves of the

Table 1. The number of leaves used for the chamber measurements and C:N measurements.

Site ID	Species	canopy position	Chamber	C:N
FAI	<i>Picea mariana</i>	overstory	8	15
	<i>Betula glandulosa</i>	understory	4	8
	<i>Ledum groenlandicum</i>	understory	3	10
	<i>Vaccinium uliginosum</i>	understory	0	5
	<i>Vaccinium vitis-idaea</i>	understory	0	7
	<i>Sphagnum capillifolium</i>	moss	1	5
	<i>Pleurozium schreberi</i>	moss	1	6
PFF	<i>Picea mariana</i>	understory	1	9
	<i>Betula papyrifera</i>	overstory	2	16
	<i>Populus tremuloides</i>	overstory	3	15
	<i>Betula glandulosa</i>	understory	0	2
	<i>Ledum groenlandicum</i>	understory	0	8
	<i>Vaccinium uliginosum</i>	understory	0	5
	<i>Vaccinium vitis-idaea</i>	understory	0	3
DLS	<i>Picea mariana</i>	overstory	2	10
	<i>Ledum groenlandicum</i>	understory	0	11
	<i>Vaccinium uliginosum</i>	understory	0	3
	<i>Vaccinium vitis-idaea</i>	understory	0	11

overstory trees (*Picea mariana*, *Betula papyrifera*, and *Populus tremuloides*), understory shrubs (*Betula glandulosa*, and *Ledum groenlandicum*), and mosses (*Sphagnum capillifolium*, and *Pleurozium schreberi*); number of the samples are shown in Table 1. The mean air temperature within the chamber ranged from 24.2°C to 36.3°C during the study period. The measurements were conducted in sunny conditions when the PPFD was greater than 680 μmol m⁻² s⁻¹.

Photosynthetic and transpiration rates for vascular plants were measured for leaves including branches, whereas those for moss samples were measured by inserting mosses into the chamber. Mosses were sampled in a transparent vessel (4.8 × 4.8 cm² of bottom area), and the photosynthetic rate under light conditions was measured. Due to the measurement approach used, the fluxes for mosses were for ground area rather than for leaf area. Dark respiration per unit of ground area could not possibly be determined using an assumption of the one-point method (shown in section 3.1), because the estimated maximum carboxylation rate was for the moss canopy instead of for the unit of leaves. Thus, the respiration rate of mosses under dark conditions was measured a few minutes after dark conditions were made.

The projected leaf area within the chamber was measured by scanning all leaves using a scanner (GT-S650, EPSON, Japan) after the chamber measurements. The needle area of *Picea mariana* was estimated based on needle length (7.56 ± 0.94 mm; n = 168), needle diameter (0.1 mm), and the number of needles (Brand, 1987; Chen *et al.*, 1997). The leaf area for mosses was assumed as unit per ground area; thus, the leaf area index (LAI) of the mosses was set to 1.0 m² m⁻².

2.4 Chemical characteristics

The C:N ratio of leaves was measured using an organic elemental analyzer (FLASH 2000, Thermo Fisher Scientific, USA) (Table 1). Leaves were dried at 60°C for two to three days after the samples were collected. Then, the dried samples were ground into a mill shape, and 4–6 mg of the samples were used for the analysis. For moss species, whole shoot system with approximately 4 ± 1 cm thickness, rather than capitulum (the apex of the *Sphagnum* shoot), were used for the chemical analysis. In addition to leaves that were measured by the chamber, leaves outside the chamber were collected from various vegetation within the three sites, and were analyzed for the C:N ratios (Table 1).

2.5 Eddy covariance measurements

For the three sites, turbulent fluxes were measured using the open-path eddy covariance method (Ueyama *et al.*, 2014a; Iwata *et al.*, 2011; Welp *et al.*, 2007). Wind velocity for the three sites was measured using same sonic anemometer (CSAT3, Campbell Scientific Inc., USA). CO₂ and water vapor densities were measured using an infrared gas analyzer (LI-7500, Li-Cor Inc., USA) for FAI and DLS, and an infrared gas analyzer (EC150, Campbell Scientific Inc., USA) for PFF. A known bias of CO₂ density by the EC150 was corrected based on Helbig *et al.* (2016). The measurement heights were 6 m at FAI, 9.5 m at DLS and 3.9 m at PFF. The turbulence data were recorded at 10 Hz using a data logger (CR3000, Campbell Scientific Inc., USA)

for FAI and PFF, and a data logger (CR5000, Campbell Scientific Inc., USA) for DLS.

3. Data analysis

3.1 Ecophysiological parameters at the leaf scale

The maximum carboxylation rate per unit of leaf area (v_c_{max}) was estimated based on the one-point method (de Kauwe *et al.*, 2016).

$$v_{c\text{max}} = (A_{\text{sat}} + R_l) \frac{(C_i + K_m)}{(C_i - \Gamma^*)} \quad (1)$$

where A_{sat} is the photosynthesis rate at saturating irradiances (800~ μmol m⁻² s⁻¹ for *Picea mariana* and *Ledum groenlandicum*; 900~ μmol m⁻² s⁻¹ for *Betula papyrifera*; 1000~ μmol m⁻² s⁻¹ for other species), C_i is the intercellular CO₂ concentration, K_m is the Michaelis-Menten constant, and Γ* is the CO₂ compensation point for photosynthesis in the absence of mitochondrial respiration. C_i was estimated from the atmospheric CO₂ concentration and stomatal conductance. Assuming a temperature dependence of K_m and Γ* (Kattge and Knorr, 2007), the maximum carboxylation at 25°C (v_c_{max25}) was estimated from v_c_{max} under different temperature conditions in the field. Stomatal conductance was estimated using the measured transpiration, leaf temperature, and vapor pressure within the chamber. R_l is the mitochondrial respiration in light, which is assumed to be 1.5% of v_c_{max} (de Kauwe *et al.*, 2016). The R_l for mosses was directly measured under dark conditions, because photosynthesis of mosses was measured per unit ground area instead per unit leaf area. When directly measured R_l instead of assuming R_l to be 1.5% of v_c_{max}, the estimated v_c_{max} decreased by 3.7% for *Sphagnum capillifolium* and 10.5% for *Pleurozium schreberi*.

The slope (m_{bb}) and intercept (b_{bb}) in the stomatal conductance model (Ball *et al.*, 1987) were determined based on the leaf chamber measurements as follows:

$$g_{sw} = m_{bb} \frac{A}{c_s} rh_s + b_{bb} \quad (2)$$

where g_{sw} is stomatal conductance for water vapor, A is the net photosynthetic rate, rh_s is the relative humidity at the leaf surface, and c_s is the CO₂ concentration at the leaf surface. The model parameters were determined for each plant, because the relationship for the same species at different sites tended to be similar, except for *Populus tremuloides*. As two distinct responses were examined for *Populus tremuloides*, two sets of m_{bb} and b_{bb} were determined.

3.2 Ecophysiological parameters at the ecosystem scale

Ecosystem-scale ecophysiological parameters were estimated based on the optimization of the iBLM-EC model (Ueyama *et al.*, 2016). The sun/shade radiation transfer (de Pury and Farquhar, 1997) was included in the current version (version 2) of the model. The model was driven with environmental and micrometeorological data, including air temperature, relative humidity, PPFD, and LAI to predict gross primary productivity (GPP) and transpiration. In this study, the model was optimized using eddy-covariance-based GPP and transpiration using the

SCE-UA method (Duan *et al.*, 1992). The optimized ecophysiological parameters were four big-leaf parameters: the maximum carboxylation rate at 25°C ($V_{C\max25}$; Farquhar *et al.*, 1980), a maximum electron transport rate at 25°C ($J_{\max25}$), and m_{bb} and b_{bb} in the stomatal conductance model (Ball *et al.*, 1987). The parameterization of the photosynthesis model was based on Kattge and Knorr (2007), which was the same parameterization as for the one-point method in section 3.1. The estimated canopy-scale parameters were then downscaled into units per leaf area ($V_{C\max25}$; values per leaf area) using LAI with radiation and nitrogen distribution factors (de Pury and Farquhar 1997; Lloyd *et al.*, 2010). Further details of the model and optimization are shown in Ueyama *et al.* (2016).

GPP and transpiration were estimated based on the eddy covariance measurements. GPP was calculated as the difference between ecosystem respiration and net ecosystem exchange (NEE), where ecosystem respiration was estimated using the Q_{10} function for air temperature (Ueyama *et al.*, 2013, 2014a). In analyzing nighttime data, we rejected data under calm conditions using a friction velocity threshold (Ueyama *et al.*, 2013). Transpiration was derived from the measured evapotranspiration; soil evaporation that was assumed as the potential evaporation at the forest floor (Ryu *et al.*, 2011) was subtracted from evapotranspiration. We did not use data under wet conditions during rain and within one hour after rain. Further details regarding data preparation for the model are shown in Ueyama *et al.* (2016).

Comparing the ecosystem-scale parameters to the leaf-scale measurements, the optimized ecosystem-scale parameters were averaged during the mid-summer period from mid-July to mid-August, when the chamber measurements were conducted. For DLS, available data from the eddy covariance measurements were limited from 2002 to 2004 (Randerson *et al.*, 2006; Welp *et al.*, 2007). We used the mean of the ecosystem-scale parameters for the mid-summer period from 2002 to 2004 for DLS.

The LAI was estimated based on field observations and satellite remote sensing. For FAI and PFF, the LAI was measured using a plant canopy analyzer (LAI-2000, Li-Cor, USA), showing 1.7 m² m⁻² for FAI and 1.9 m² m⁻² for PFF from mid-July to mid-August (Ueyama *et al.*, 2014a; Iwata *et al.*, 2013). The LAI for DLS was estimated based on a relationship between enhance vegetation index (EVI) derived from the Moderate Resolution Spectroradiometer (MODIS) and the LAI at FAI, which was 2.5 m² m⁻² from mid-July to mid-August (Ueyama *et al.*, 2016).

Comparing the leaf-scale $V_{C\max25}$ with ecosystem-scale $V_{C\max25}$ derived from the model optimization, the leaf-scale $V_{C\max25}$ was upscaled to scales of overstory and understory using the leaf C:N ratio. Based on a significant linear relationship between the leaf C:N ratio and measured $V_{C\max25}$ (shown in section 4.1), the ecosystem-scale overstory and understory $V_{C\max25}$ were calculated using the leaf C:N ratio measured for overstory and understory species at each site. The dominant overstory for FAI and DLS was *Picea mariana*, whereas the dominant overstory species for PFF were *Betula papyrifera* and *Populus tremuloides*.

4. Results

4.1 Leaf scale measurement

Across vascular plants, the measured $V_{C\max25}$ was lowest for *Picea mariana*, and the $V_{C\max25}$ values for *Betula papyrifera* and *Populus tremuloides* were the highest (Fig. 1; Table 2). The values of $V_{C\max25}$ for the other two understory species (*Betula glandulosa* and *Ledum groenlandicum*) were in the middle of these two ranges. The values of $V_{C\max25}$ for mosses (*Sphagnum capillifolium* and *Pleurozium schreberi*) were lower than those for the vascular plants.

The variations in the measured $V_{C\max25}$ of the different vascular plants were explained by the leaf C:N ratios ($R^2 = 0.68$, $p < 0.01$, $n = 18$; Fig. 1a), except for one *Picea mariana* sample.

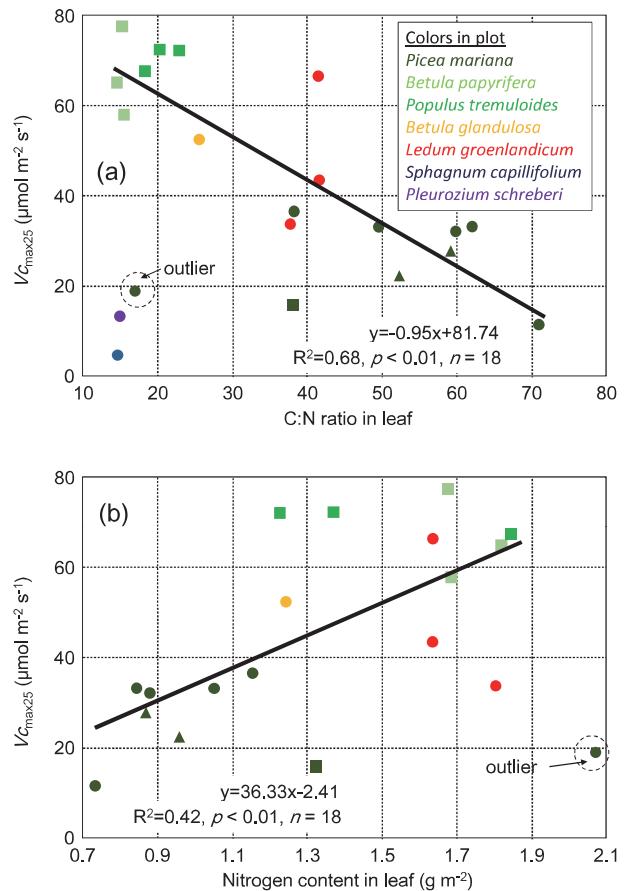


Fig. 1. Relationship of leaf C:N ratio (a) and leaf nitrogen content (b) to the maximum carboxylation rate at 25°C ($V_{C\max25}$) measured by a leaf chamber across two mature black spruce forests and one aspen-birch forest during mid-summer in interior Alaska. The $V_{C\max25}$ for vascular plants are per unit of leaf area, whereas those for mosses are per unit of ground area. The regression lines are shown for vascular plants except one outlier. Different colors represent different species. The dot represents data for FAI, the square represents data for PFF, and the triangle represents data for DLS. Note that specific leaf area for calculating leaf nitrogen content was from previous literatures rather than our study sites (Table 2). The number of the data points was less than those in Table 1, due to an incompatibility among chamber and leaf C:N measurements.

The relationship to the leaf C:N ratio was clearer than that to leaf nitrogen content (Fig. 1b), where the leaf nitrogen content was estimated from the leaf C:N ratios using specific leaf area from literatures (Bond-Lamberty *et al.*, 2002; Kloeppe *et al.*, 1998; Table 2). The low vc_{max25} in *Picea mariana* was explained by high C:N ratio, whereas the high vc_{max25} values in *Betula papyrifera* and *Populus tremuloides* were explained by the low C:N ratio. In contrast, the measured vc_{max25} values for mosses were lower than those expected from the relationship.

4.2 Ecosystem scale comparison

The ecosystem-scale vc_{max25} from the eddy covariance data was comparable to the upscaled leaf-scale vc_{max25} using leaf C:N ratios (Fig. 2). The ecosystem-scale vc_{max25} was similar to values of understory vc_{max25} for FAI (open canopy forest), reflecting greater contributions of the understory to canopy photosynthesis. In contrast, the ecosystem-scale vc_{max25} for DLS was smaller than the vc_{max25} of the overstory and understory

for this site (closed canopy forest). This result was possibly due to overestimates of the input LAI for the model, because the LAI in DLS was estimated using satellite data. The ecosystem-scale vc_{max25} was similar to the understory vc_{max25} in PFF. This result indicates that the understory greatly contributed to canopy photosynthesis in the early succession at PFF. Among the three sites, the estimated understory vc_{max25} was similar ($39\text{--}47 \mu\text{mol m}^{-2} \text{s}^{-1}$), although the canopy structure and vegetation composition differed.

Stomatal conductance parameters, m_{bb} , were compared among the leaf chamber and ecosystem-scale estimations (Table 2). Based on the chamber measurement, the m_{bb} ranged from 5.7 to 15.6 in FAI (Table 2), which were consistent with values for the ecosystem scale (10.0). The optimized m_{bb} was greater than those for overstory by the leaf scale measurements in DLS and PFF. A precise comparison could not be made due to lack of measurements for the understory in the DLS and PFF sites.

Table 2. Measured maximum carboxylation rate standardized at 25°C (vc_{max25} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), slope (m_{bb}) and intercept (b_{bb} ; $\text{mol m}^{-2} \text{s}^{-1}$) of the stomatal conductance model (Ball *et al.*, 1987), C:N ratio in leaves that were measured by the chamber, specific leaf area (SLA; $\text{m}^2 \text{kg}^{-1}$ dry weight), nitrogen content (N_a ; g m^{-2}), carbon content per dry weight (C_w ; g g^{-1}), C:N ratio in leaves of all samples whose photosynthesis were measured and not measured by the chamber, and upscaled vc_{max25} based on the relationship examined in Fig. 1 and that predicted by the iBLM-EC model.

Site	Species / Model	canopy position	vc_{max25}	m_{bb} ^{*a}		b_{bb} ^{*a}		C:N ^{*b}	N_a ^{*c}	SLA ^{*c}	C_w (%) ^{*d}	C:N ^{*d}	vc_{max25} ^{*e}							
				mean	stdev	mean	stdev													
FAI	<i>Picea mariana</i>	overstory	26.2	9.6	5.7	0.4	0.02	0.00	49.6	19.5	1.1	0.5	9.76	48.7	6.7	58.5	20.3	28.7	14.4	
	<i>Betula glandulosa</i>	understory	58.3	6.4	7.2	2.1	0.07	0.04	26.3	1.1	1.2	0.1	15.55	48.6	2.3	30.0	7.7	49.0	5.5	
	<i>Ledum groenlandicum</i>	understory	47.8	16.8	15.6	3.0	0.02	0.02	40.3	2.2	1.7	0.1	7.82	52.9	0.6	42.0	4.9	40.5	3.5	
	<i>Vaccinium uliginosum</i>	understory	--	--	--	--	--	--	--	--	--	--	--	49.8	0.7	33.8	12.0	49.1	2.3	
	<i>Vaccinium vitis-idaea</i>	understory	--	--	--	--	--	--	--	--	--	--	--	51.3	0.5	56.6	10.3	30.1	7.3	
	<i>Sphagnum capillifolium</i>	moss	4.9	--	45.3	13.3	-0.02	0.02	14.7	--	4.9	--	--	48.7	1.1	16.6	3.5	--	--	
	<i>Pleurozium schreberi</i>	moss	5.1	--	-- ^{*g}	-- ^{*g}	-- ^{*g}	-- ^{*g}	15.0	--	5.1	--	--	54.9	8.0	18.8	9.0	--	--	
	Canopy (iBLM-EC)	--	38.1	4.6	10.0	4.6	0.04	0.11	--	--	--	--	--	--	--	--	44.7	9.1	--	--
PFF	<i>Picea mariana</i>	understory	15.8	--	5.7	0.4	0.0	0.0	38.1	--	1.3	--	9.76	50.1	0.6	39.6	5.7	42.2	4.1	
	<i>Betula papyrifera</i>	overstory	66.7	9.9	6.7	1.4	0.02	0.05	15.1	0.5	1.7	0.1	18.48	51.6	5.8	17.6	6.5	57.8	4.6	
	<i>Populus tremuloides</i>	overstory	70.5	2.7	5.5,	0.9,	0.05,	0.03,	20.5	2.3	1.5	0.3	17.42	50.2	7.1	19.6	4.5	56.4	3.2	
	<i>Betula glandulosa</i>	understory	--	--	--	--	--	--	--	--	--	--	--	48.4	3.7	17.4	3.1	57.9	2.2	
	<i>Ledum groenlandicum</i>	understory	--	--	--	--	--	--	--	--	--	--	--	53.4	0.8	30.7	1.7	48.5	1.2	
	<i>Vaccinium uliginosum</i>	understory	--	--	--	--	--	--	--	--	--	--	--	47.9	2.8	33.8	12.0	47.7	3.1	
	<i>Vaccinium vitis-idaea</i>	understory	--	--	--	--	--	--	--	--	--	--	--	51.9	0.3	45.0	11.5	38.3	8.2	
	Canopy (iBLM-EC)	--	40.7	7.9	18.2	9.3	0.13	0.08	--	--	--	--	--	--	--	--	42.5	7.9	-	-
DLS	<i>Picea mariana</i>	overstory	25.1	3.8	5.7	0.4	0.0	0.0	55.7	4.8	0.9	0.1	9.76	51.9	3.0	54.0	6.9	31.9	4.9	
	<i>Ledum groenlandicum</i>	understory	--	--	--	--	--	--	--	--	--	--	--	52.6	1.1	32.3	7.2	47.4	5.1	
	<i>Vaccinium uliginosum</i>	understory	--	--	--	--	--	--	--	--	--	--	--	50.9	1.5	33.8	12.0	39.3	17.5	
	<i>Vaccinium vitis-idaea</i>	understory	--	--	--	--	--	--	--	--	--	--	--	51.0	3.7	46.6	8.2	37.2	5.8	
	Canopy (iBLM-EC) ^{*f}	--	15.0	4.2	17.8	5.9	0.06	0.10	--	--	--	--	--	--	63.9	3.5	--	--	--	

^{*a} Parameters were determined for each plant, because the relationship for the same species at different sites tended to be similar, except for *Populus tremuloides*.

^{*b} Leaf C:N ratios for leaves that the photosynthesis measurement was conducted.

^{*c} SLA data from Kloeppe *et al.* (1998) for *Picea mariana* and Bond-Lamberty *et al.* (2002) for other species was used for calculating N_a .

^{*d} The mean of all samples that were used and not used for the photosynthesis measurements.

^{*e} Based on the leaf C:N ratio, the vc_{max25} predicted using a relationship between the leaf C:N ratio and measured vc_{max25} (Fig. 1).

^{*f} The eddy covariance data from the past study (Welp *et al.*, 2007) from 2002 to 2004 were used.

^{*g} No significant relationship was obtained.

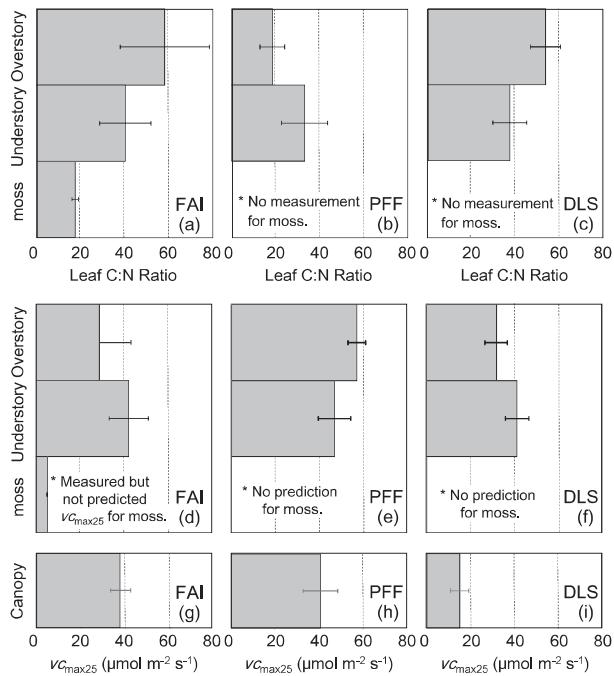


Fig. 2. Leaf C:N ratio (a, b, c) and maximum carboxylation rate at 25°C (vc_{max25}) upscaled using the relationship examined in Fig. 1a and the leaf C:N ratio (d, e, f) for overstory and understory at the three forests in interior Alaska. The optimized ecosystem-scale vc_{max25} was from the eddy covariance data using the iBLM-EC model (g, h, i). The value of vc_{max25} for moss (d) was the measured value rather than predicted value using the relationship in Fig. 1a. Error bars in a-f represent the standard deviations for different plants, whereas those for g-i are the standard deviations in vc_{max25} during the period from mid-July to mid-August.

5. Discussion

The leaf C:N ratio, thus leaf nitrogen content, was strongly related to the carboxylation capacity at the leaf, and thus to the photosynthetic capacity under light saturation conditions (Fig. 1). The relationship indicates that the nitrogen cycle was strongly coupled to the carbon cycle in boreal forests (Clein *et al.*, 2002; Euskirchen *et al.*, 2010). The strong linearity was previously examined at the global scale (Kattge *et al.*, 2009), where nitrogen content had a linear relationship to vc_{max} . The strong linearity indicates that increases in N availability associated with high-latitude warming (Chapin *et al.*, 2005) potentially stimulate photosynthesis (Euskirchen *et al.*, 2010; Ueyama *et al.*, 2009). Although the linearity was stronger in using C:N ratio than nitrogen content, superiority of C:N ratio is not conclusive because specific leaf area for calculating nitrogen content was referred from previous literatures.

The inter- and within-species variations in leaf C:N ratio and vc_{max25} showed that each plant had a small within-species variability of leaf C:N ratio and vc_{max25} (Fig. 1). Despite different soil characteristics, such as the burned upland (PFF) and bog on permafrost (FAI), the variations in vc_{max25} of *Picea mariana* among ecosystems were smaller than the inter-species variation. The small within-species variability of the photosynthetic capacity in *Picea mariana* infers that the potential

increases in vc_{max} of *Picea mariana* could be small due to their small plasticity, even if N availability increases. This result may be consistent with a finding that recent warming decreased the radial ring growth of *Picea mariana* trees (Beck *et al.*, 2011), which could be caused by competitive advantage of productive understory plants. The decreased contribution in *Picea mariana* could increase the ecosystem-scale vc_{max25} due to increased contributions of the understory.

The small within-species variability of the leaf C:N ratio and vc_{max25} suggests that the future increase in canopy photosynthesis due to increased N availability could be associated with changes in canopy structure and species composition. Increased N availability could favor a productive understory rather than increased photosynthesis of *Picea mariana*, as discussed above. Changes in species composition in adapting to higher nutrient environments were reported as an important process in arctic tundra (Shaver *et al.*, 2001). The structural changes could influence canopy photosynthesis in addition to changes in the LAI (McMillan and Goulden, 2008).

The carboxylation capacity of mosses was considerably lower than those expected from low C:N ratio (Fig. 1). This was because allocation of nitrogen to photosynthetic tissues differed to those for vascular plants (Rice *et al.*, 2008). Rice *et al.* (2008) reported that nitrogen content only for capitulum correlated to photosynthetic rates; we measured C:N ratio for whole shoot system rather than capitulum. The high C:N ratios for mosses were partly because the vc_{max25} values for mosses were measured per unit of ground area instead of per unit of leaf area; photosynthesis in low moss layers did not possibly meet light saturation conditions.

The measured leaf-scale parameters, vc_{max25} , and m_{bb} , were comparable to previous studies at boreal forests in North America. The measured vc_{max} for *Picea mariana* were within the range of those previously reported at a non-permafrost soil in Canada (8.5~50 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Dang *et al.*, 1998; Cai and Dang, 2002). The measured vc_{max} were smaller than those measured at a temperate region in Wisconsin, USA for *Betula papyrifera* (84 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Uddling *et al.*, 2009) and *Populus tremuloides* (76 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Uddling *et al.*, 2009), but similar to those reported at a non-permafrost soil, Canada (50~60 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Cai and Dang, 2002). The values for *Sphagnum* and *Pleurozium* mosses (5~14 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Williams and Flanagan, 1998) were consistent with our study, but a higher vc_{max} of 78 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was reported for *Ledum groenlandicum* in Canada (Bubier *et al.*, 2011). The values of m_{bb} were within a range reported in previous measurements (4~16; Ball *et al.*, 1987; Medlyn *et al.*, 2011), except for mosses that do not have stomata and passively control gas diffusion according to different water conditions (Williams and Flanagan, 1998).

Steps toward improving the canopy photosynthesis model were suggested in this study. First, the measured difference in nitrogen content and vc_{max25} between overstory and understory differed from the concept that the photosynthetic capacity was positively correlated to light availability within a canopy (e.g., Lloyd *et al.*, 2010). Greater photosynthetic capacity of the understory could be characteristic of boreal forests in interior Alaska (Ikawa *et al.*, 2015). Modification of the vertical nitrogen

distribution factor (Lloyd *et al.*, 2010) for boreal forests could improve the predictive accuracy of the model. Second, the values of m_{bb} should be evaluated with the leaf- and ecosystem-scale measurements. The measured leaf-scale m_{bb} differed among the plants, and the estimated ecosystem-scale m_{bb} varied among the ecosystems (Table 2). Upscaling m_{bb} considering within-canopy variations improves the predictive accuracy of the model. Further leaf-scale measurements of m_{bb} , including moss species and development of the upscaling method, are required in future studies. Evaluating the water use efficiency at the leaf and ecosystem scales could help provide precise estimates of CO₂ and water vapor fluxes.

6. Conclusion

Evaluating the ecophysiological parameters via ecosystem- and leaf-scale measurements was useful for improving canopy photosynthesis models and understanding the ecophysiology in boreal forests. The ecosystem-scale photosynthetic parameters were influenced by canopy structure and species composition, such as different contributions of the overstory and understory within a canopy. This finding indicates that appropriate modeling of overstory and understory improves the accuracy in simulations of the carbon cycle in boreal forests in interior Alaska. The strong linkage between the leaf C:N ratio and photosynthetic capacity also indicates that modeling the nitrogen cycle is important for predicting the carbon cycle. A precise understanding is necessary to evaluate how the canopy structure and species composition change with increased N availability due to high-latitude warming.

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