

# Net ecosystem CO<sub>2</sub> exchange for moss and lichen dominated forest floors of old-growth sub-boreal spruce forests in central British Columbia, Canada

Rachel S. Botting<sup>a</sup>, Arthur L. Fredeen<sup>a,b,\*</sup>

<sup>a</sup> *Ecosystem Science and Management Program, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada*

<sup>b</sup> *Natural Resources and Environmental Studies Institute, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada*

Received 10 February 2006; received in revised form 13 August 2006; accepted 13 August 2006

## Abstract

This study used instantaneous chamber-based CO<sub>2</sub> exchange measurements (2004) in conjunction with a seasonal record of microclimate (2003) to model growing season forest floor net ecosystem CO<sub>2</sub> exchange (ffNEE) for terrestrial bryophyte and lichen communities in sub-boreal forests in central British Columbia, Canada. Multiple regression models using microclimate variables described between 35 and 53% of the variation in ffNEE for moss or lichen dominated forest floor at an ambient CO<sub>2</sub> concentration. Light and moss or lichen moisture and temperature were all important variables in describing ffNEE from moss and lichen dominated forest floor patches while substrate temperature was the most important variable explaining ffNEE from bare litter + soil and wood. Moss dominated forest floor had relatively invariant mean diel ffNEE across the 3-month growing season while lichen dominated wood had low summer ffNEE which increased in September. Over a 3-month growing season in 2003, moss dominated forest floor had a total ffNEE of  $-33.8 \text{ g C m}^{-2}$  and lichen dominated wood had a total ffNEE of  $-42.9 \text{ g C m}^{-2}$ . When ffNEE values from the moss, lichen, bare wood, and bare litter + soil components of the forest floor community were summed over the 3-month period, the old-growth sub-boreal spruce forest floor had a net CO<sub>2</sub> exchange of  $-31.6 \text{ g C m}^{-2}$ , representing a loss of this amount of carbon over the growing season. The moss dominated, but not lichen dominated, forest floor appeared limited by ambient forest floor CO<sub>2</sub> levels ( $430 \mu\text{mol CO}_2 \text{ mol}^{-1}$ ) and exhibited increased photosynthesis at elevated CO<sub>2</sub> ( $700 \mu\text{mol CO}_2 \text{ mol}^{-1}$ ).

© 2006 Elsevier B.V. All rights reserved.

**Keywords:** Bryophyte; Lichen; Sub-boreal spruce forest; Old-growth; Ecosystem CO<sub>2</sub> exchange; Photosynthesis

## 1. Introduction

In light of the continued increases in greenhouse gases, such as CO<sub>2</sub>, in the Earth's atmosphere (Keeling and Whorf, 2005) and the associated global warming (Prentice et al., 2001) there is a growing need to identify and quantify sources and sinks for CO<sub>2</sub> globally. Among terrestrial systems, forests are particularly significant with boreal forests alone containing nearly a quarter of all terrestrial vegetation and soil carbon (Watson et al., 2000) but with poorly understood overall CO<sub>2</sub> source or

sink status (Malhi et al., 1999) due to both high spatial and interannual variability (Amiro et al., 2006).

Bryophytes and lichens are common forest floor components of many ecosystems, particularly northern conifer forests, however, they are commonly overlooked in carbon budget models. Recently it has been shown that the boreal terrestrial bryophyte and lichen community can contribute significantly to the overall forest CO<sub>2</sub> fluxes (Goulden and Crill, 1997; Morén and Lindroth, 2000; Swanson and Flanagan, 2001). For example, mosses may take up 35% of the forest floor CO<sub>2</sub> efflux in Boreal forests (Swanson and Flanagan, 2001) and can store 10–50% of the gross CO<sub>2</sub> uptake in black spruce forests (Goulden and Crill, 1997). Thus, a better understanding of the net CO<sub>2</sub> exchange from other forest floor communities may contribute significantly to our understanding of forest carbon cycling and forest responses to future climate change.

\* Corresponding author at: Ecosystem Science and Management Program, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada. Tel.: +1 250 960 5847.

E-mail address: [freedena@unbc.ca](mailto:freedena@unbc.ca) (A.L. Fredeen).

In mosses and lichens, photosynthesis and respiration are controlled largely by microclimate conditions (Palmqvist, 2000). Due to their poikilohydric nature, the growth of bryophytes and lichens is often limited by environmental water availability and a lack of moisture may frequently restrict photosynthesis (Hahn et al., 1993; Sundberg et al., 1997; Palmqvist and Sundberg, 2000). Optimal water contents for photosynthesis vary by species with moss and lichens. In the arctic, *Peltigera* species reached maximum photosynthesis rates at water contents of 250% (Lange et al., 1996) while the moss *Hylocomnium splendens* reached maximum photosynthesis at water contents ranging from 300 to 700% (Sonesson et al., 1992). With sufficient moisture, light and temperature are frequently limiting factors (Hahn et al., 1993; Palmqvist, 2000; Heijmans et al., 2004). Light levels in the forest understory are often patchy due to a variable canopy overhead, with short sunflecks creating periods of elevated light in the otherwise shaded understory (Percy and Pfitsch, 1995; Canham et al., 1999) and providing a high proportion of the total light intensity (Chazdon and Percy, 1991). As well, irradiance decreases steeply with depth in the moss layer, indicating that only the top layer of a moss mat is likely to be photosynthetically active (DeLucia et al., 2003). Respiration from the underlying soil and woody substrates of the forest floor is largely affected by temperature and moisture availability (e.g. Russell and Voroney, 1998; Drewitt et al., 2002; Dilustro et al., 2005).

In a world experiencing increasing atmospheric CO<sub>2</sub> concentrations, mosses and lichens on the forest floor are already growing in a localized, elevated CO<sub>2</sub> environment due to their proximity to the respiring soil layer below (Sonesson et al., 1992; Tarnawski et al., 1994; Green and Lange, 1995; Coxson and Wilson, 2004). For example, Coxson and Wilson (2004) found average CO<sub>2</sub> levels of 700 μmol mol<sup>-1</sup> in the middle of moss mats and 430 μmol mol<sup>-1</sup> at mat surfaces in a sub-alpine spruce forest. This elevated CO<sub>2</sub> environment may affect bryophyte photosynthesis, potentially increasing productivity. Some moss species appear to be CO<sub>2</sub> limited at ambient levels and do not become CO<sub>2</sub> saturated until 2000 μmol mol<sup>-1</sup> (Green and Lange, 1995). There is less consensus on the effect that elevated CO<sub>2</sub> has on lichen species, partly due to the varying responses of some lichens to elevated CO<sub>2</sub> and the dependence of the response on moisture content (Green and Lange, 1995; Lange et al., 1996). It is not certain what effect steadily increasing atmospheric CO<sub>2</sub> will have on terrestrial bryophytes and lichens in sub-boreal forests.

Forest floor net ecosystem CO<sub>2</sub> exchange (ffNEE) estimates across a growing season have commonly been obtained using chamber-based measurements, either with continuously operated automated chamber systems (e.g. Goulden and Crill, 1997) or manually operated instantaneous flux measurements (e.g. Swanson and Flanagan, 2001), with the latter requiring some form of modeling and continuous seasonal microclimate data for temporal scaling. The latter approach was used in this study to assess the *in situ* contributions of bryophyte and lichen forest floor communities to the CO<sub>2</sub> exchange of sub-boreal forests. Instantaneous ffNEE measurements were made across the 2004 growing season under natural light or dark conditions for moss

or lichen dominated forest floor and for bare litter + soil or wood substrates. Instantaneous microclimate measurements, made in conjunction with the instantaneous ffNEE measurements in 2004, were used to generate multiple regression models that were then applied to continuous seasonal microclimate measurements made in 2003 to model ffNEE over an entire growing season.

The present study had two primary objectives: (1) to measure and then model the specific contributions of bryophytes and lichens to overall forest floor CO<sub>2</sub> exchange in sub-boreal spruce forest and (2) to assess the effect of elevated CO<sub>2</sub> level (700 μmol mol<sup>-1</sup>) on moss and lichen ffNEE and CO<sub>2</sub>-exchange processes in relation to ambient CO<sub>2</sub> (430 μmol mol<sup>-1</sup>) at the forest floor (Coxson and Wilson, 2004).

## 2. Methods

### 2.1. Study area

This study was conducted in the Aleza Lake Research Forest (ALRF) approximately 60 km northeast of Prince George, in central British Columbia, Canada (122°40'W, 54°11'N). The ALRF is located in the Sub-Boreal Spruce biogeoclimatic zone in the cool wet variant SBSwk1 (Meidinger and Pojar, 1991). The dominant tree species at the ALRF are hybrid spruce (*Picea glauca* (Moench) Voss × *engelmannii* Parry) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) with Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco), trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marsh.) making up lesser proportions of the canopy (DeLong, 2003). At an elevation of 600–700 m, the climate is characterized by cool snowy winters and moist cool summers (OES, 1995). The ALRF receives 900 mm of precipitation a year, with 65% of that falling as rain and 35% falling as snow, and has average monthly temperatures ranging from about 20 °C in July to –20 °C in January (Murphy, 1996). Soils in the region consist primarily of fine-textured, clay dominated glaciolacustrine soils with scattered pockets of overlying coarse-textured soils (Arocena and Sanborn, 1999).

Two replicate forest stands were examined in this study, both of which were located in old-growth sub-boreal spruce forest (>200 years of age) growing on the more commonly observed fine textured soils. At both study sites, mosses, liverworts, and lichens constituted on average 53% of the forest floor cover with mosses comprising the majority of this cover (Botting and Fredeen, 2006). The most common moss species included *Pleurozium schreberi* (Brid.) Mitt., *Rhytidiadelphus triquetrus* (Hedw.) Warnst., *Ptilium crista-castrensis* (Hedw.) DeNot. and *H. splendens* (Hedw.) B.S.G while the most common lichens were of the genus *Peltigera* Willd. This study examined the moss *R. triquetrus* growing on soil substrate and the lichen *Peltigera membranacea* (Ach.) Nyl. growing on coarse woody debris (CWD) substrate. *R. triquetrus* is a suberect moss typically growing in loose mats on the litter layer (Schofield, 1992), while *P. membranacea* is a foliose lichen with a cyanobacterial photobiont often found growing on decaying

wood (Brodo et al., 2001). The CWD substrates studied were all of a moderate decay class, decay class 3–4 using definitions taken from the British Columbia Ministry of Forests (where 1 is less decayed and 5 is most decayed) (Ministry of Forests and Ministry of Environment, 1998).

## 2.2. Seasonal microclimate measurements

During the 2003 growing season, microclimate stations were set up at the study sites from 26 June to 22 October and data loggers (Campbell Scientific, Logan, UT, USA) recorded microclimate data every 5 min. At one site, photon flux density (PFD) measurements were recorded from three quantum sensors (Li-Cor Inc., Lincoln, NE, USA) randomly placed across the forest floor. At both sites, air and soil temperatures were measured, the latter using copper constantan thermocouples (Omega Engineering Inc., Indianapolis, IN, USA) inserted 10 cm into the ground. The temperature of lichen thalli and moss fronds were measured using fine wire copper constantan thermocouples (Omega Engineering Inc.) affixed in the middle of moss mats or through lichen thalli.

The moisture contents of three lichen thalli and three moss fronds at each site were measured using the impedance method as described by Coxson (1991). Electrical impedance measurements were taken between pairs of non-serrated, 1 mm wide microclips (also known as alligator clips) attached to the outer edge of a lichen thallus or the main stem of a moss frond, at a distance of 5 mm apart. The microclips were covered with plastic clip covers to prevent interference with current flow. An AC half bridge with excitation voltage of 2500 mV was applied to the microclips from the data logger and impedance was measured in ohms. In the lab, impedance measurements were calibrated to actual lichen and moss percent moisture content (calculated as (wet weight – dry weight)/dry weight  $\times$  100). Percent moisture values were then plotted against impedance values and fit with non-linear functions that were used to compute moss frond or lichen thallus percent moisture content over the growing season. The relationship for lichens was given by  $y = 100 \times (0.9952 + 310.53/x)$  and for mosses was given by  $y = 100 \times \exp(-0.1811 + 117.406/x)$ , where  $y$  is percent moisture content and  $x$  is impedance in ohms.

Equipment malfunctions resulted in some data gaps in the 2003 microclimate measurements. Gaps in moisture and temperature data from one microclimate site were filled using data from the corresponding period from the other site as they were only 6 km apart and experienced similar climatic conditions. As only one site had quantum sensors, small quantum data gaps were filled using average light values from days on either side of the gap. One larger gap was filled by first assessing each day's approximate light level from a climate station in the research forest and then filling the gap with data from days with similar light levels.

## 2.3. Forest floor CO<sub>2</sub> concentrations

Coxson and Wilson (2004) found mean CO<sub>2</sub> levels of 430  $\mu\text{mol mol}^{-1}$  at the surface of forest floor moss mats and

700  $\mu\text{mol mol}^{-1}$  mid moss mat in sub-alpine spruce fir forests in Alberta. Based on these results, 430  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  was taken to represent the ambient CO<sub>2</sub> level at the forest floor and 700  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  was taken to represent elevated CO<sub>2</sub>.

On several occasions during May and early June 2004, the CO<sub>2</sub> concentration in forest floor moss mats was measured with the LI6400 Portable Photosynthesis System (Li-Cor Inc.) using 2 m of flexible Excelon Bev-A-Line tubing (Thermoplastic Processes Inc., Stirling, NJ, USA) with pin prick holes along the length. A low flow rate was used to minimize the sampling of ambient air above the forest floor layer. The CO<sub>2</sub> concentration in the middle of the living moss mat layer averaged 439  $\mu\text{mol mol}^{-1}$  and reached a maximum concentration of 520  $\mu\text{mol mol}^{-1}$ . The diel CO<sub>2</sub> concentration at the surface of the moss mat averaged 387  $\mu\text{mol mol}^{-1}$ .

## 2.4. Moss and lichen collars

Twenty plastic PVC collars (diameter 10 cm; depth 5 cm) were installed in pairs at intervals over each of the two forest sites for a total of 40 collars. The collars were installed 1 week prior to the commencement of measurements to minimise disturbance effects on flux measurements. Pairs of collars were sunk 3 cm into the substrate with the first collar located in either a homogenous area of the moss *R. triquetrus* growing on soil or over a large lichen thallus, *P. membranacea*, growing on coarse woody debris. The second collar was installed on adjacent soil or wood from which the mosses and lichens had been removed down to bare wood or bare litter. If any vascular plants occurred inside the collars, the plant and its roots were carefully removed at the time of collar installation.

## 2.5. Instantaneous net ecosystem CO<sub>2</sub> exchange measurements

Instantaneous forest floor net ecosystem CO<sub>2</sub> exchange (ffNEE;  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) is defined, in the light, as the sum of moss or lichen photosynthesis and respiration plus litter, soil, wood, heterotrophic, and root respiration. In the dark, it is the sum of moss, lichen, litter, soil, wood, heterotrophic, and root respiration. In this paper, negative ffNEE values are used to indicate a loss of CO<sub>2</sub> to the atmosphere or net ecosystem respiration and positive ffNEE values are used to indicate uptake of CO<sub>2</sub> from the atmosphere or net ecosystem photosynthesis.

Instantaneous ffNEE measurements were made between 17 May and 27 September 2004, typically between 9:00 a.m. and 5:00 p.m., using an open flow LI6400 Portable Photosynthesis System (Li-Cor Inc.). A custom chamber was constructed using 3 mm thick plexi-glass lined with Teflon tape and the Li-Cor custom chamber kit (Li6400-19, Li-Cor Inc.). The cylindrical chamber was 10 cm (diameter) by 17 cm (height) with a chamber volume of 1135 cm<sup>3</sup> and a basal surface area of 78.5 cm<sup>2</sup>. The bottom of the chamber had an overlapping lip, which fit snugly down over the PVC collars. During operation, the fan inside the sensor head circulated air from the chamber into the IRGA in the sensor head with the flow rate set at

500  $\mu\text{mol s}^{-1}$  and the fan speed set at high. No internal chamber fan was required because of the relatively small chamber volume (LI6400 Application Note #3, Li-Cor Inc.). Relative humidity inside the chamber was constrained to a maximum of 80% and normally never went below 45%. Similar chamber based Li-Cor gas-exchange approaches have been used by others for measurement of small scale ecosystem level fluxes (e.g. Vourlitis et al., 1993; Swanson and Flanagan, 2001).

Pairs of collars were visited on average 5.4 times each for a total of 107 visits to the 20 pairs of collars over the 3-month season. After the chamber was attached to a collar, and after each change in  $\text{CO}_2$  concentration, an equilibration period of at least 5 min was permitted for the chamber to come within 95% of the  $\text{CO}_2$  concentration set point (LI6400 Application Note #3, Li-Cor Inc.). At the moss or lichen collars, measurements were first taken at a  $\text{CO}_2$  concentration of 430  $\mu\text{mol mol}^{-1}$  in the light and then with the chamber darkened using a black shade cloth. With the chamber still covered, measurements were taken at a  $\text{CO}_2$  concentration of 700  $\mu\text{mol mol}^{-1}$ . The cloth was then removed, a period of reacclimation to light conditions occurred, and measurements were taken in the light at 700  $\mu\text{mol mol}^{-1}$ . The chamber was moved to the adjacent bare wood or bare litter + soil collar and measurements were taken in the light at  $\text{CO}_2$  concentrations of 430 and 700  $\mu\text{mol mol}^{-1}$ , respectively. For all measurements, ffNEE values were allowed to stabilize before three points were logged at 10 s intervals and averaged.

At the time of each ffNEE measurement, moss frond or lichen thallus temperature was measured using a fine wire chromega constantan thermocouple (Omega Engineering Inc.) inside the chamber, coupled to the Li6400 sensor head. External air temperature was measured by the LI6400 and an external quantum sensor (LI9901-013, Li-Cor Inc.) mounted on the sensor head measured PFD. At the start of a measurement sequence, moss frond or lichen thallus moisture was measured on a comparable specimen growing adjacent to the collar using the impedance measurement technique discussed above. As soil temperature was not measured at this time, air temperature was used to estimate soil temperature using a relationship ( $r^2 = 0.78$ ) between air and soil temperature derived from the 2003 seasonal microclimate data. Throughout the field season, efforts were made to take measurements under a full range of moisture, light, and temperature conditions.

## 2.6. Modeling seasonal NEE

Multiple linear regressions were generated, relating instantaneous ffNEE and simultaneous microclimate measurements from 2004. These regression relationships then permitted the prediction of a seasonal ffNEE profile using the continuous seasonal microclimate data of 2003. Input regression variables included moss frond and lichen thallus temperature, soil temperature, moss frond and lichen thallus moisture content, and mean forest floor PFD. The PFD variable was log transformed to provide a linear relationship with NEE and to improve normality. No other microclimate variable was transformed before analysis. Backwards elimination was used

to select variables with an  $\alpha$  level of 0.10 as a threshold for inclusion of a microclimate variable in a regression equation. Collinearity and multicollinearity among independent variables were assessed and only those with tolerance scores  $< 0.2$  were accepted as explanatory variables. The ffNEE measurements from the two study sites were pooled before analysis to increase sample size. All multiple regression models were significant at the  $\alpha = 0.05$  level.

Multiple regression equations were created for moss or lichen dominated forest floor in the light and dark at  $\text{CO}_2$  concentrations of 430 and 700  $\mu\text{mol mol}^{-1}$  for a total of four equations each. Bare wood or litter + soil multiple regression equations were created at  $\text{CO}_2$  concentrations of 430 and 700  $\mu\text{mol mol}^{-1}$  for two equations each.

Before the regression equations could be applied to the 2003 seasonal microclimate data, the data had to be divided into light and dark periods. A threshold light level of  $\text{PFD} = 5 \mu\text{mol m}^{-2} \text{s}^{-1}$  was determined to be a suitable light level below which most mosses and lichens would be respiring and below their respective light compensation points. Light compensation points of 12–20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for a cyanobacterial *Peltigera* (Lange et al., 1996) and 5–10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for two foliose lichen species (Sundberg et al., 1997) have been reported. Sonesson et al. (1991) found a light compensation point of 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the moss *H. splendens*. In this paper, ffNEE in the light is denoted as ffNEE<sub>L</sub> and in the dark as ffNEE<sub>D</sub>.

## 3. Results

### 3.1. Influence of microclimate on instantaneous ffNEE

Moisture, light, and temperature all affected forest floor ffNEE rates over the growing season. PFD was highest in July and August (average 45  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and decreased through September (average 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) while, conversely, moss frond and lichen thallus moisture levels were lowest in July and August and increased through September (Table 1). Mosses and lichens were dry 32–50% of the time in July and August but only 8% (mosses) and 14–18% (lichens) of the time in September. Moss frond and lichen thallus temperatures were slightly higher in August than July and decreased by an average of 3 °C in September. Temperatures were consistently higher during periods when the mosses and lichens were dry.

Some general relationships between instantaneous ffNEE and microclimate were observed. For example, low moss frond moisture resulted in negative ffNEE while at high moisture levels, positive ffNEE was curtailed primarily by low light levels ( $\text{PFD} < 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (data not shown). There was a trend towards less positive moss ffNEE at high temperatures, however, this was also when low moisture was commonly observed. In lichen dominated forest floor, positive ffNEE values were curtailed by low moisture levels and, at high moisture levels, were limited by low light levels ( $\text{PFD} < 20 \mu\text{mol m}^{-2} \text{s}^{-1}$  at lower temperatures to  $\text{PFD} < 40 \mu\text{mol m}^{-2} \text{s}^{-1}$  at higher temperatures). Both wood and litter + soil showed increased respiration at higher temperatures and exhibited the lowest ffNEE values at the lowest recorded temperatures. The

Table 1  
Mean microclimate conditions at the forest floor of a sub-boreal spruce forest over a 3-month period in 2003, measured for the moss *R. triquetrus* and the lichen *P. membranacea* in the light and the dark

	27 June–26 July	27 July–25 August	26 Aug–24 September
<b>Light</b>			
Moss temperature (°C) <sup>a</sup>	16.0	16.7	11.4
Lichen temperature (°C)	15.6	16.4	11.3
Soil temperature (°C) <sup>b</sup>	12.2	12.2	10.0
PFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) <sup>c</sup>	45.6	45.3	30.9
Moss moisture (%) <sup>d</sup>	152	151	238
Lichen moisture (%)	211	144	226
Time moss spent dry (%) <sup>e</sup>	42	48	8
Time lichen spent dry (%) <sup>f</sup>	32	50	18
<b>Dark</b>			
Moss temperature (°C)	12.0	11.9	8.6
Lichen temperature (°C)	12.0	11.8	8.5
Soil temperature (°C)	11.4	11.8	10.2
PFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0.7	0.7	0.6
Moss moisture (%)	140	140	224
Lichen moisture (%)	208	147	226
Time moss spent dry (%)	42	46	8
Time lichen spent dry (%)	32	45	14

Note: Light is defined as periods of time with a PFD > 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  while dark is defined as all periods with lesser PFD values.

<sup>a</sup> Moss/lichen temperature refers to the moss frond or lichen thallus temperature.

<sup>b</sup> Soil temperature refers to the temperature at 10 cm depth in the soil.

<sup>c</sup> PFD is the photosynthetic flux density.

<sup>d</sup> Moss/lichen moss refers to the percent moisture content of the moss frond or lichen thallus.

<sup>e</sup> Dry was defined as moss frond moisture contents below 90%.

<sup>f</sup> Dry was defined as lichen thallus moisture contents below 110%.

relationship between ffNEE and moisture was not as clear in wood and litter + soil substrates.

Climate data from a permanent climate station at the research forest indicated that the climate was relatively similar between the 2003 and 2004 seasons (1 June–1 October). Average temperatures over this period varied by only 0.5 °C between years (12.6 °C in 2003 versus 13.1 °C in 2004). Rainfall varied slightly between the 2 years with a greater number of small rainfall events and total amount in 2004 compared with fewer,

larger rainfall events and a lower total amount in 2003 (263 mm in 2003 versus 327 mm in 2004). Total growing season solar radiation varied by <2% across years.

### 3.2. Comparison of modeled and measured instantaneous ffNEE at ambient CO<sub>2</sub>

All of the ffNEE multiple regression models were significant at  $\alpha = 0.05$  (Table 2). At a CO<sub>2</sub> concentration of

Table 2  
Multiple regression equations for the estimation of net ecosystem CO<sub>2</sub> exchange (ffNEE) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for lichen (*P. membranacea*) dominated wood, moss (*R. triquetrus*) dominated forest floor, bare soil + litter (litter), and bare wood substrates in the light and dark and at CO<sub>2</sub> concentrations of 430 and 700  $\mu\text{mol mol}^{-1}$  in a sub-boreal spruce forest

	<i>n</i>	Regression equation	<i>R</i> <sup>2</sup>	<i>F</i>	<i>p</i>
<b>CO<sub>2</sub> = 430 <math>\mu\text{mol mol}^{-1}</math></b>					
Lichen (light)	51	ffNEE <sub>L</sub> = 1.35 log <i>A</i> – 0.06 <i>B</i> + 0.14 <i>C</i> – 1.86	0.48	14.18	<0.001
Lichen (dark)	47	ffNEE <sub>D</sub> = –0.038 <i>B</i> – 0.11 <i>C</i> + 0.22	0.47	19.47	<0.001
Moss (light)	54	ffNEE <sub>L</sub> = 1.41 log <i>A</i> – 0.04 <i>B</i> + 0.06 <i>C</i> – 1.43	0.53	19.47	<0.001
Moss (dark)	53	ffNEE <sub>D</sub> = –0.10 <i>C</i> – 0.517	0.35	27.32	<0.001
Litter (light and dark)	54	ffNEE = –0.089 <i>D</i> – 0.11 <i>C</i> + 0.91	0.38	15.41	<0.001
Wood (light and dark)	47	ffNEE = –0.0397 <i>D</i> + 0.182	0.14	7.10	0.011
<b>CO<sub>2</sub> = 700 <math>\mu\text{mol mol}^{-1}</math></b>					
Lichen (light)	51	ffNEE <sub>L</sub> = 1.54 log <i>A</i> – 0.05 <i>B</i> + 0.13 <i>C</i> – 2.24	0.32	7.38	<0.001
Lichen (dark)	51	ffNEE <sub>D</sub> = –0.02 <i>B</i> – 0.09 <i>C</i> – 0.17	0.23	7.17	0.002
Moss (light)	53	ffNEE <sub>L</sub> = 2.22 log <i>A</i> – 0.09 <i>B</i> – 1.46	0.67	51.60	<0.001
Moss (dark)	53	ffNEE <sub>D</sub> = –0.11 <i>C</i> – 0.46	0.38	31.82	<0.001
Litter (light & dark)	52	ffNEE = –0.0758 <i>D</i> – 0.104 <i>C</i> + 0.758	0.34	12.42	<0.001
Wood (light and dark)	51	ffNEE = –0.0487 <i>D</i> + 0.286	0.14	7.67	0.008

Note: Variables include: *A* = PFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), *B* = moss frond or lichen thallus temperature (°C), *C* = moss frond or lichen thallus moisture (proportion), and *D* = soil temperature (°C). The significance value for inclusion of variables in the regression equations was *p* = 0.1. Forest floor NEE in the light is denoted ffNEE<sub>L</sub> and is defined as periods of time with a PFD > 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  while ffNEE in the dark is denoted ffNEE<sub>D</sub> and is defined as all periods with lesser PFD values.

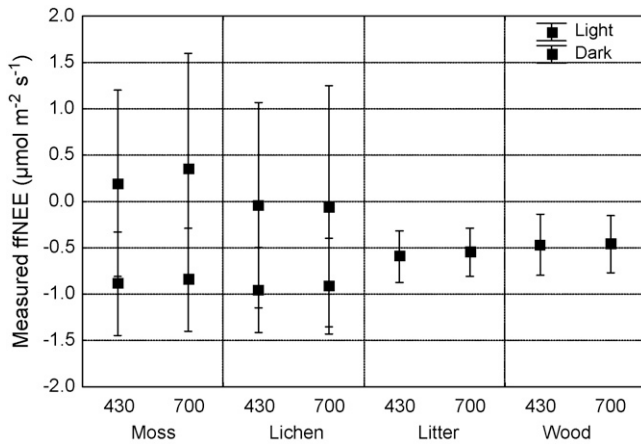


Fig. 1. Mean ( $\pm$ standard deviation) measured instantaneous forest floor net ecosystem  $\text{CO}_2$  exchange (ffNEE) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) measured over the 2004 growing season for moss and lichen dominated forest floor, bare litter + soil (litter), and bare wood in the light and dark at  $\text{CO}_2$  concentrations of 430 and 700  $\mu\text{mol CO}_2 \text{mol}^{-1}$ . Note that light is defined as periods of time with PFD  $> 5 \mu\text{mol m}^{-2} \text{s}^{-1}$  while dark is all periods with lesser PFD values. Litter and wood measurements were made only in the light at the two  $\text{CO}_2$  concentrations.

430  $\mu\text{mol mol}^{-1}$ , the lichen regression equations included PFD, thallus temperature, and thallus moisture in the light ( $R^2 = 0.48$ ), and thallus temperature and moisture in the dark ( $R^2 = 0.47$ ). The moss regression equations included PFD, frond temperature, and frond moisture variables in the light ( $R^2 = 0.53$ ) and included only frond moisture in the dark ( $R^2 = 0.35$ ). Moss, bare wood, and bare litter + soil all had similar  $R^2$  values at 430  $\mu\text{mol mol}^{-1}$  and 700  $\mu\text{mol mol}^{-1}$  while lichen systems had higher  $R^2$  values at 430  $\mu\text{mol mol}^{-1}$ .

Moss dominated forest floor had higher measured instantaneous mean diel ffNEE values (0.2–0.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) than lichen dominated wood ( $-0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Fig. 1). Bare wood and litter + soil had similar measured instantaneous mean ffNEE values. Modeled instantaneous ffNEE values for moss or lichen dominated forest floors were similar to measured values with respect to minimum values, but underestimated mean and maximum values (data not shown). Measured instantaneous mean diel ffNEE for moss dominated forest floors was  $+0.196 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 1) while modeled ffNEE ranged from  $+0.038$  to  $-0.062 \mu\text{mol m}^{-2} \text{s}^{-1}$  across the growing season. For lichen dominated wood, measured instantaneous mean diel ffNEE was  $-0.039 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 1) compared to modeled values, which ranged from  $-0.422$  to  $-0.652 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Graphical comparisons of measured and modeled instantaneous ffNEE rates showed a good fit to the 1:1 line for lichen in the light and dark and for moss in the light (Fig. 2). Moss ffNEE in the dark showed a poorer fit due to the dependence of that regression model solely on moss moisture content which was constrained by measurable maximum and minimum moisture contents.

### 3.3. Trends in modeled monthly and seasonal ffNEE at ambient $\text{CO}_2$

When modeled over the 3-month season, trends in ffNEE could be observed. At a  $\text{CO}_2$  concentration of 430  $\mu\text{mol mol}^{-1}$ ,

lichen dominated forest floor had more negative modeled seasonal ffNEE ( $-42.96 \text{ g C m}^{-2}$ ) than moss dominated forest floor ( $-33.86 \text{ g C m}^{-2}$ ) (Table 3). Moss and lichen dominated forest floors also differed dramatically in their light ffNEE and dark ffNEE. Moss ffNEE<sub>L</sub> was consistently much less negative than moss ffNEE<sub>D</sub>. Over the growing season, moss ffNEE<sub>L</sub> was relatively close to zero ( $-0.54 \text{ g C m}^{-2}$ ), indicating that moss photosynthesis was on average balancing below ground and moss respiration in the light. Conversely, lichen ffNEE<sub>L</sub> and ffNEE<sub>D</sub> values were both negative and of similar magnitudes. Only during the third month was lichen ffNEE<sub>L</sub> less negative than ffNEE<sub>D</sub>.

Bare litter + soil and wood had comparable modeled seasonal ffNEE values with total growing season ffNEE being  $-28.56 \text{ g C m}^{-2}$  for bare litter + soil and  $-25.52 \text{ g C m}^{-2}$  for wood (Table 3). Both showed a trend towards more negative ffNEE<sub>L</sub> early in the season, with more hours of daylight, and less negative ffNEE<sub>L</sub> later in the season.

### 3.4. Effect of elevated $\text{CO}_2$ concentration

Increasing the measurement  $\text{CO}_2$  concentration from 430 to 700  $\mu\text{mol CO}_2 \text{mol}^{-1}$  had a positive effect on moss net photosynthesis. Measured instantaneous mean diel ffNEE increased from  $0.196 \mu\text{mol m}^{-2} \text{s}^{-1}$  (430  $\mu\text{mol CO}_2 \text{mol}^{-1}$ ) to  $0.355 \mu\text{mol m}^{-2} \text{s}^{-1}$  (700  $\mu\text{mol CO}_2 \text{mol}^{-1}$ ). Over the 3-month growing season, moss seasonal ffNEE ranged from an average of  $-33.8 \text{ g C m}^{-2}$  (430  $\mu\text{mol CO}_2 \text{mol}^{-1}$ ) to  $-15.3 \text{ g C m}^{-2}$  (700  $\mu\text{mol CO}_2 \text{mol}^{-1}$ ) (Table 3). Moss seasonal ffNEE<sub>D</sub> was almost constant between the two  $\text{CO}_2$  concentrations while moss seasonal ffNEE<sub>L</sub> increased substantially from 430 to 700  $\mu\text{mol CO}_2 \text{mol}^{-1}$ . The elevated  $\text{CO}_2$  concentration allowed for an apparent increase in *R. triquetrus* photosynthesis in the light but, as expected, had no impact on dark respiration.

In contrast, lichen photosynthesis was not obviously affected by  $\text{CO}_2$  concentration. Measured instantaneous mean diel ffNEE decreased slightly from 430 to 700  $\mu\text{mol mol}^{-1}$ . Lichen seasonal ffNEE and seasonal ffNEE<sub>L</sub> and ffNEE<sub>D</sub> values were, in fact, all slightly less positive at the higher  $\text{CO}_2$  concentration (Table 3).

### 3.5. Moss and lichen photosynthesis and respiration

Mean moss and lichen diel net photosynthesis, nocturnal respiration, gross photosynthesis, and net carbon gain, independent of the substrate below, were estimated over the growing season (Table 4) from measured and modeled instantaneous ffNEE measurements. At 430  $\mu\text{mol CO}_2 \text{mol}^{-1}$ , moss gross photosynthesis was  $+1.08 \mu\text{mol m}^{-2} \text{s}^{-1}$  based on measured ffNEE and  $+0.68 \mu\text{mol m}^{-2} \text{s}^{-1}$  based on modeled ffNEE values (Table 4). Lichen gross photosynthesis estimated from measured ffNEE values was  $+0.92 \mu\text{mol m}^{-2} \text{s}^{-1}$  while modeled gross photosynthesis was much lower at  $-0.10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . For both mosses and lichens, modeled values were lower than measured values. Modeled and summed over the season, gross photosynthesis

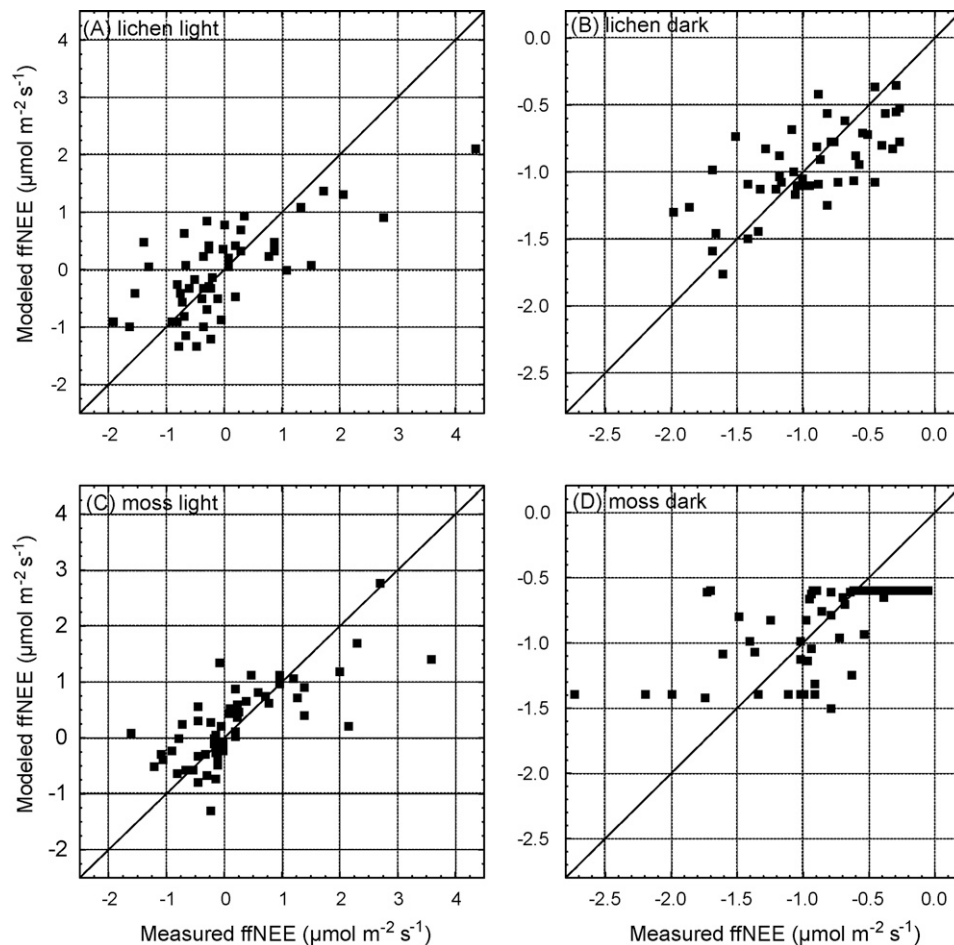


Fig. 2. Comparison of instantaneous measured and modeled forest floor net ecosystem CO<sub>2</sub> exchange (ffNEE) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for lichen (*P. membranacea*) and moss (*R. triquetrus*) dominated forest floor in the light and the dark. All figures are for an ambient CO<sub>2</sub> concentration of 430  $\mu\text{mol mol}^{-1}$  with 1:1 lines shown. Correlation coefficients were (a) 0.69, (b) 0.69, (c) 0.73 and (d) 0.59 and all correlations were significant at  $\alpha = 0.05$ .

Table 3  
Modeled net ecosystem CO<sub>2</sub> exchange (ffNEE) (430 and 700  $\mu\text{mol CO}_2 \text{mol}^{-1}$ ) for moss or lichen dominated forest floor, bare litter + soil (litter), or bare wood for 3 months individually ( $\text{g C m}^{-2} \text{month}^{-1}$ ), totalled seasonally over day (ffNEE<sub>L</sub>) and night (ffNEE<sub>D</sub>) ( $\text{g C m}^{-2} \text{season}^{-1}$ ), and totalled over the 2003 season ( $\text{g C m}^{-2} \text{season}^{-1}$ ) in a sub-boreal spruce forest

	ffNEE 27 June–26 July ( $\text{g C m}^{-2} \text{mo}^{-1}$ )	ffNEE 27 July–25 August ( $\text{g C m}^{-2} \text{mo}^{-1}$ )	ffNEE 26 August–24 September ( $\text{g C m}^{-2} \text{mo}^{-1}$ )	Seasonal ffNEE <sub>L</sub> /ffNEE <sub>D</sub> ( $\text{g C m}^{-2} \text{season}^{-1}$ )	Seasonal total ffNEE ( $\text{g C m}^{-2} \text{season}^{-1}$ )
Moss light (430)	-0.08	-0.95	0.50	-0.54	
Moss dark (430)	-9.51	-10.34	-13.47	-33.32	-33.86
Moss light (700)	6.06	4.06	5.61	15.74	
Moss dark (700)	-8.82	-9.58	-12.68	-31.08	-15.34
Lichen light (430)	-8.21	-10.08	-5.54	-23.83	
Lichen dark (430)	-6.70	-6.16	-6.28	-19.14	-42.96
Lichen light (700)	-9.76	-11.42	-7.32	-28.48	
Lichen dark (700)	-8.78	-8.62	-9.95	-27.36	-55.84
Litter light (430)	-5.67	-5.38	-3.65	-14.70	
Litter dark (430)	-3.62	-5.09	-5.15	-13.86	-28.56
Litter light (700)	-5.36	-5.08	-3.62	-14.06	
Litter dark (700)	-3.52	-4.82	-5.06	-13.40	-27.46
Wood light (430)	-5.04	-4.70	-3.01	-12.74	
Wood dark (430)	-3.77	-4.68	-4.32	-12.77	-25.52
Wood light (700)	-5.14	-4.78	-2.88	-12.81	
Wood dark (700)	-3.73	-4.77	-4.18	-12.68	-25.50

Note: Light is defined as periods of time with PFD > 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  while dark is defined as all periods with lesser PFD values.

Table 4

Seasonal ( $\text{g C m}^{-2} \text{ season}^{-1}$ ) and instantaneous mean ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) diel net photosynthesis (PS), nocturnal net respiration (Resp.), gross photosynthesis (PS), and net carbon gain (at  $\text{CO}_2$  concentrations of 430 and 700  $\mu\text{mol mol}^{-1}$ ) for the moss and lichen components of the forest floor after mathematically removing  $\text{CO}_2$  exchange from underlying litter, soil, and wood substrates

	Derived moss or lichen respiration and photosynthesis			
	Diel net PS <sup>a</sup>	Nocturnal net resp. <sup>b</sup>	Gross PS <sup>c</sup>	Net carbon gain <sup>d</sup>
Instantaneous measured ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )				
Moss (430)	0.79	-0.29	1.08	0.50
Moss (700)	0.88	-0.31	1.19	0.57
Lichen (430)	0.43	-0.49	0.92	-0.06
Lichen (700)	0.41	-0.45	0.86	-0.05
Instantaneous modeled ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )				
Moss (430)	0.31	-0.38	0.68	-0.08
Moss (700)	0.63	-0.36	0.99	0.27
Lichen (430)	-0.19	-0.13	-0.10	-0.32
Lichen (700)	-0.37	-0.31	-0.06	-0.68
Seasonal modeled ( $\text{g C m}^{-2} \text{ season}^{-1}$ ) <sup>e</sup>				
Moss (430)	14.17	-18.61	32.78	-4.44
Moss (700)	29.80	-17.86	46.82	12.12
Lichen (430)	-11.09	-6.37	-4.69	-17.46
Lichen (700)	-15.67	-14.68	-1.12	-30.35

<sup>a</sup> Mean diel net moss or lichen photosynthesis = [mean growing season moss or lichen  $\text{ffNEE}_L$  - mean growing season litter or wood  $\text{ffNEE}$ ].

<sup>b</sup> Mean nocturnal net moss or lichen respiration = [mean growing season moss or lichen  $\text{ffNEE}_D$  - mean growing season litter or wood  $\text{ffNEE}$ ].

<sup>c</sup> Mean moss or lichen gross photosynthesis = [mean growing season moss or lichen  $\text{ffNEE}_L$  - mean growing season moss or lichen  $\text{ffNEE}_D$ ].

<sup>d</sup> Averaged over the 3 months, approximately 49% of the hours in a day were light and 51% were dark, as previously defined. Therefore, Net moss or lichen carbon gain = [mean diel net photosynthesis + mean net nocturnal respiration].

<sup>e</sup> Seasonal modeled values are derived from seasonal  $\text{ffNEE}$  (Table 3) which are instantaneous  $\text{ffNEE}$  values modeled every 5 min and then summed over the 3-month season.

was 32.79  $\text{g C m}^{-2}$  for mosses and -4.69  $\text{g C m}^{-2}$  for lichens, at 430  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ . Net carbon gain over the 3 months was negative for both mosses (-4.44  $\text{g C m}^{-2}$ ) and lichens (-17.46  $\text{g C m}^{-2}$ ).

### 3.6. Seasonal $\text{ffNEE}$ from sub-boreal spruce forest floors

The proportion of the forest floor covered by each of moss, lichen, bare wood, and bare litter + soil was used to scale  $\text{ffNEE}$  over the landscape. A concurrent study (Botting and Fredeen, 2006), found the average percent cover of bryophytes and lichens in old-growth sub-boreal spruce forest on fine textured soils to be 53% (2% lichen, 51% moss and liverwort) while coarse woody debris had 10% cover and bare litter + soil covered the remaining 37% of the area. Tree basal areas comprised 0.5% of the area and for the purposes of this calculation were omitted. When these percent cover estimates were multiplied by the respective seasonal  $\text{ffNEE}$  values (Table 3) and summed over the moss, lichen, bare wood, and bare litter components of the forest floor community, the old-growth sub-boreal spruce forest floor lost -31.6  $\text{g C m}^{-2}$  over the 3-month period (Fig. 3).

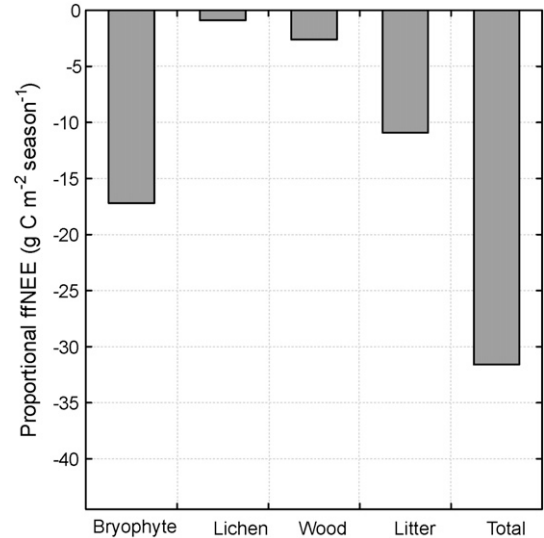


Fig. 3. Proportional forest floor net ecosystem  $\text{CO}_2$  exchange ( $\text{ffNEE}$ ) ( $\text{g C m}^{-2} \text{ season}^{-1}$ ) over a 3-month growing season for the bryophyte, lichen, bare wood, and bare litter + soil components of old-growth sub-boreal spruce forest floor. Note that proportional  $\text{ffNEE}$  was calculated using the seasonal  $\text{ffNEE}$  and the proportional forest floor cover of 51% moss, 2% lichen, 10% bare wood, and 37% bare litter cover.

## 4. Discussion

### 4.1. Microclimate influences on forest floor $\text{ffNEE}$

Photosynthesis in both moss and lichen dominated forest floors was limited to periods of adequate frond or thallus moisture and then further to periods of sufficient light and temperature. This was particularly the case for lichens with the maximum measured instantaneous diel  $\text{ffNEE}$  measurement (4.3  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) occurring during a time of very high moisture content (800%), high PFD (1300  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), and high temperature (26 °C). These conditions did not occur frequently. That the photosynthesis of mosses and lichens is limited by adequate light, and by sufficient moisture due to their poikilohydric nature, has been observed in many studies (e.g. Hahn et al., 1993; Sundberg et al., 1997; Palmqvist and Sundberg, 2000). In this study, the productivity of the moss and lichen forest floors was constrained by both moisture and light conditions experienced during the growing season.

### 4.2. Moss and lichen $\text{ffNEE}$ , photosynthesis, and respiration

Measured instantaneous  $\text{ffNEE}$  values ranged from -2.7 to +3.6  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  for moss dominated forest floor and -2.0 to +4.4  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  for lichen dominated forest floor. These results are somewhat consistent with those of Swanson and Flanagan (2001) for boreal feather moss systems where net  $\text{CO}_2$  exchange ranged from -5 to +1  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ . Forest floor respiration values recorded in this study (-2.7 to -0.1  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) were at the end of the range of soil only respiration values (-6 to -2  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) found for mature



forest at the Aleza Lake Research Forest (Pypker and Fredeen, 2003), but were similar to those observed by Goulden and Crill (1997) for feather moss dominated boreal black spruce forests where night time CO<sub>2</sub> efflux from the forest floor ranged between  $-2.5$  and  $-1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ . As would be expected, the forest floor flux values from this study are bracketed by the range of whole-forest fluxes observed for mature boreal forest stands across Canada ( $-8$  to  $+8 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Coursolle et al., 2006).

The individual flux contributions of mosses and lichens (photosynthesis, respiration, carbon gain) were derived to assess their independent contributions to ecosystem level CO<sub>2</sub> fluxes. Mosses, after subtraction of soil and litter fluxes, had positive mean gross photosynthetic rates of  $+1.08 \mu\text{mol m}^{-2} \text{s}^{-1}$  (measured) and  $+0.68 \mu\text{mol m}^{-2} \text{s}^{-1}$  (modeled). Lichens had lower mean gross photosynthesis than mosses;  $+0.92 \mu\text{mol m}^{-2} \text{s}^{-1}$  (measured) and  $-0.10 \mu\text{mol m}^{-2} \text{s}^{-1}$  (modeled). Other studies have reported maximum photosynthetic rates of  $+1.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  for the feather moss *P. schreberi* in a boreal forest (Whitehead and Gower, 2001) and  $+1.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  for moss mats taken from the forest floor of a temperate rainforest in New Zealand (DeLucia et al., 2003). Goulden and Crill (1997) observed gross photosynthesis to range from  $+0.5$  to  $+1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  for boreal feather mosses, similar to our results.

In both moss and lichen, modeled values were more negative than measured values of ffNEE, net daytime photosynthesis, gross photosynthesis, and net carbon gain. For example, measured instantaneous net carbon gain for mosses over the season was approximately  $+0.50 \mu\text{mol m}^{-2} \text{s}^{-1}$ , suggesting mosses had a gain of carbon over the 3-month period, while modeled instantaneous net carbon gain was  $-0.08 \mu\text{mol m}^{-2} \text{s}^{-1}$  and seasonal modeled net carbon gain was  $-4.44 \text{ g C m}^{-2}$  suggesting a small loss of carbon. These lower modeled values may have occurred due to the moss and lichen models underestimating ffNEE rates, leading to lower estimates of gross photosynthesis and net carbon gain over the season. Alternatively, it may be that the moss and lichen measured ffNEE values overestimate ffNEE due to the fact that the instantaneous measured values were all taken under relatively favourable climatic conditions and during the middle part of the day. Further, the low growth rates of bryophytes and lichens in the low light understory environment mean that net carbon gain is close to zero making it difficult to accurately measure these small values using this approach.

#### 4.3. Patterns in moss and lichen dominated forest floor ffNEE

Seasonal ffNEE patterns indicate that mosses and lichens may have different photosynthetically active periods over the growing season. The moss, *R. triquetrus*, seemed to be consistently photosynthetically active through the summer and fall and exhibited relatively invariant photosynthetic activity over the season while lichens showed enhanced photosynthesis in the fall. For all 3 months, moss seasonal ffNEE<sub>L</sub> was close to zero, indicating a balance between moss photosynthesis and

respiration, while ffNEE<sub>D</sub> became progressively more negative across the season as night length increased. By contrast, lichen dominated wood had more negative ffNEE<sub>L</sub> values than ffNEE<sub>D</sub> values during the first 2 months (27 June–26 August) and September was the only month in which lichen ffNEE<sub>L</sub> was less negative than ffNEE<sub>D</sub>. This change was even more surprising given that September is the month in our study with the least number of photosynthetic hours. As well, both measured and modeled net carbon gain for lichens implied that over the 3-month period, the lichens lost carbon. These patterns may be due to seasonal ecosystem conditions. Forest floor and CWD moisture levels typically increase in late summer and early fall in accordance with lower light levels, lower temperatures, and less transpiration, particularly after leaf-fall. Increased time spent in a hydrated state in late summer and early fall (Table 1) would be expected to favour photosynthetic CO<sub>2</sub> uptake by lichen photobionts. It may be that *P. membranacea* was most photosynthetically active in the fall and possibly in the spring (before our measurements began). Heijmans et al. (2004) found a similar trend where *Sphagnum* moss was photosynthetically active through the summer season while lichen and the moss *H. splendens* lost CO<sub>2</sub> in the middle of the growing season and increased CO<sub>2</sub> uptake again at the end of the growing season as moisture content increased. A prolonged measurement period, extending further into the spring and fall would be required to determine if this pattern is occurring.

#### 4.4. Effects of elevated CO<sub>2</sub> concentration

Preliminary analysis in this study showed that an elevated CO<sub>2</sub> environment occurred in the moss layer at the forest floor of sub-boreal spruce forests. The average CO<sub>2</sub> concentration in the moss mats ( $439 \mu\text{mol mol}^{-1}$ ) was higher than both the CO<sub>2</sub> level at the surface of the moss mats ( $387 \mu\text{mol mol}^{-1}$ ) and the global average CO<sub>2</sub> concentration of  $377 \mu\text{mol mol}^{-1}$  (Keeling and Whorf, 2005). These CO<sub>2</sub> levels are similar to those found in a temperate rainforest in New Zealand where the CO<sub>2</sub> concentration was  $466 \mu\text{mol mol}^{-1}$  in the top layer of the moss mats and  $376 \mu\text{mol mol}^{-1}$  10 cm above the moss mats (DeLucia et al., 2003). *H. splendens* moss mats in the sub-arctic had average CO<sub>2</sub> concentrations of  $400$ – $450 \mu\text{mol mol}^{-1}$  (Sonesson et al., 1992). However, this study did not find the highly elevated CO<sub>2</sub> environments shown to occur in moss mats in some other areas (Tarnawski et al., 1994; Coxson and Wilson, 2004). Additional examination would be required to gain a more complete picture of the CO<sub>2</sub> environment in sub-boreal moss mats.

In this study, the moss *R. triquetrus* seemed to be CO<sub>2</sub> limited at 'ambient' forest floor levels ( $430 \mu\text{mol mol}^{-1}$ ) and photosynthesis was considerably enhanced by increasing the CO<sub>2</sub> concentration to  $700 \mu\text{mol mol}^{-1}$ . Mean measured moss gross photosynthesis at a CO<sub>2</sub> level of  $700 \mu\text{mol mol}^{-1}$  was  $1.19 \mu\text{mol m}^{-2} \text{s}^{-1}$ , representing a 10% increase in photosynthesis over that observed at  $430 \mu\text{mol mol}^{-1}$  and a 14% increase in measured net carbon gain. Modeled gross photosynthesis values showed a 45% increase in gross

photosynthesis. Van Der Heijden et al. (2000) observed a 17% increase in *Sphagnum* dry mass production over 6 months with an elevated CO<sub>2</sub> concentration of 700 μmol mol<sup>-1</sup>. Other studies have indicated even greater increases in photosynthesis including a three- to four-fold increase in photosynthesis observed in the moss *H. splendens* between 350 and 1000 μmol mol<sup>-1</sup> (Sonesson et al., 1992). Sonesson et al. (1992) suggested that the higher CO<sub>2</sub> levels found at the forest floor are needed in order for moss growing in the light and moisture limited environment on the forest floor to maintain positive fluxes. Increased CO<sub>2</sub> concentrations appear to positively influence photosynthesis in mosses growing under light and moisture limited conditions on the forest floor.

Photosynthesis in the lichen *P. membranacea* was not positively influenced by increased CO<sub>2</sub> concentration. Both measured and modeled gross photosynthesis at 700 μmol mol<sup>-1</sup> resulted in a slight decrease in photosynthesis at the higher CO<sub>2</sub> concentration. The literature shows there to be variation in the reported CO<sub>2</sub> dependencies of lichens (Green and Lange, 1995). In many lichen species, the CO<sub>2</sub> saturation point depends upon the diffusion resistance of CO<sub>2</sub> at different moisture contents (Lange et al., 1996). Therefore, Lange et al. (1996) have suggested that increasing atmospheric CO<sub>2</sub> concentrations may primarily increase lichen productivity during periods of high moisture saturation. The fact that the lichens in this study were moisture limited during much of the 3-month study period may have meant that increased CO<sub>2</sub> concentration had little effect on *Peltigera* photosynthesis. Alternately, while all attempts were made to achieve full acclimation between measurement conditions, it may be that the operational equilibration periods allowed before measurements were not long enough to allow for CO<sub>2</sub> equilibration in the thallus and/or for complete acclimation between dark and light transitions. Further study with the addition of controlled moisture and temperature conditions would facilitate a better understanding of the dependence of photosynthesis on CO<sub>2</sub> concentration in *P. membranacea*.

#### 4.5. Forest floor seasonal ffNEE

The growing season modeled ffNEE for the entire forest floor community [moss + lichen + litter + soil + wood] was -31.6 g C m<sup>-2</sup>. This is a less negative CO<sub>2</sub> flux than those found by studies in other forest types. For example, over a 5-month season Swanson and Flanagan (2001) found a net exchange of -255 g C m<sup>-2</sup> for the forest floor of a boreal black spruce forest and Marra and Edmonds (1994) observed soil and wood CO<sub>2</sub> fluxes of -48 and -33 g C m<sup>-2</sup> per month, respectively, in a temperate rainforest. The less negative ffNEE values observed in this study may be due to the sites being upland sites in a relatively dry climate and so having drier site conditions than either black spruce forests or temperate rainforests. As well, the old-growth stands in this study are likely near equilibrium with slower growth than may be expected in younger, more dynamic stands. In addition, the percent coverage of moss and lichen was lower in these stands

(53%) (Botting and Fredeen, 2005) than has been observed in black spruce forests with almost complete moss cover (Swanson and Flanagan, 2001).

The contributions of forest floor bryophytes and lichens to forest gross primary productivity have been estimated for ecosystems ranging from boreal black spruce forests where mosses contributed 13% of the forest gross primary productivity (Swanson and Flanagan, 2001) to temperate rainforests in New Zealand where bryophytes constituted 5% of forest gross primary productivity (DeLucia et al., 2003). Though gross primary productivity could not be calculated in this study, the average biomass carbon from the terrestrial moss and lichen layer for these old-growth forest stands was 110 ± 22 g C m<sup>-2</sup> (Botting, 2005), constituting only 0.7% of total old-growth forest biomass carbon and equivalent to 14% of conifer tree needle biomass (Botting, 2005, Fredeen et al., 2005). Thus, while bryophyte and lichen forest floor communities contribute to sub-boreal forest carbon dynamics, they are likely restricted relative to the proportion tree foliage biomass by resource limitations, particularly the transience of forest floor moisture, as well as their lower maximal values for net photosynthesis. However, it may be that the contribution of *Peltigera* species to forest floor carbon gain, though small in absolute and relative terms, could have greater implications for N<sub>2</sub>-fixation (since they contain a cyanobacterial symbiont), particularly as it would relate to the decomposition of nitrogen-poor logs on which these lichens predominantly grow.

#### 4.6. Model fit

The regression models based on moisture, light, and temperature described about 50% of the variation in CO<sub>2</sub> flux from lichen and moss dominated forest floor communities in sub-boreal British Columbia. Some of the additional variability in the moss and lichen regression models was likely due to limitations in the sensitivity of the moss frond/lichen thallus moisture measurement methodology which is less responsive to changes in moisture at the wet end of the moisture scale and is limited by its ability to detect moisture variation beyond a threshold at the dry end of the moisture scale (Coxson, 1991). Additional variation in the models may have been due to within and between site heterogeneity. Drewitt et al. (2002) noted substantial differences in flux values, even over small areas, between collars in a Douglas-fir forest, and Rayment and Jarvis (2000) similarly found considerable spatial heterogeneity in soil fluxes. Heterogeneity in the woody substrates was also likely, and may have resulted from differences in species composition. Respiration rates can vary between log species (Marra and Edmonds, 1994) and although most of the logs were spruce, it was not always possible to determine the species composition of logs.

Though statistically significant, the litter and wood regression models explained only 15–35% of the variation in ffNEE. Temperature and moisture have generally been found to be the most important factors controlling soil and wood respiration (Bowden et al., 1998; Russell and Voroney, 1998;

Drewitt et al., 2002; Marra and Edmonds, 1994). However, the use of moss and lichen moisture as surrogates for actual soil and wood moisture measurements likely reduced the explanatory power of the models. For example, lichen moisture levels are likely much more variable over the season than wood, which can retain more moisture over the summer and have less seasonal variation in moisture than either forest floor or soil (Marra and Edmonds, 1994). Had wood temperature or moisture or soil moisture measures been available, there may have been improved model fit.

It was not possible to make gross photosynthesis measurements on a finer temporal scale due to an apparent hysteresis and possible artefactual discrepancy between concurrent instantaneous measurements of moss/lichen forest floor ffNEE values and corresponding bare wood/litter + soil ffNEE values. Removal of the moss layer may have resulted in a more variable soil temperature and moisture regime because of the lost insulation layer (Oechel and Van Cleve, 1986) and reduced barrier to evaporative soil water losses and gains (Swanson and Flanagan, 2001). Bare wood may have been similarly affected by the removal of lichens and, additionally, the lichens may have been an important source of nitrogen for decomposition (Rayner and Boddy, 1988; Knowles, 2004). We would recommend the use of moss and lichen ‘plugs’ to cover bare soil + litter and wood between measurements, respectively, to minimise these microclimatic hystereses and artefacts and/or changes in nutrient inputs to the substrates.

## 5. Conclusion

Instantaneous chamber-based CO<sub>2</sub> exchange measurements in conjunction with seasonal microclimate data were used to model growing season ffNEE of the terrestrial bryophyte and lichen community in old-growth sub-boreal spruce forest. Lichen and moss moisture and temperature and forest floor light levels all had important effects on forest floor CO<sub>2</sub> exchange. Multiple regression models using microclimate variables described between 35 and 53% of the variation in moss and lichen dominated forest floor ffNEE at ambient CO<sub>2</sub> concentrations.

Measured instantaneous ffNEE values ranged from +3.6 to −2.7 μmol m<sup>−2</sup> s<sup>−1</sup> for moss dominated forest floor and +4.4 to −2.0 μmol m<sup>−2</sup> s<sup>−1</sup> for lichen dominated forest floor. Gross photosynthesis ranged between +1.08 μmol m<sup>−2</sup> s<sup>−1</sup> (measured) and +0.68 μmol m<sup>−2</sup> s<sup>−1</sup> (modeled) for mosses and +0.92 μmol m<sup>−2</sup> s<sup>−1</sup> (measured) and −0.10 μmol m<sup>−2</sup> s<sup>−1</sup> (modeled) for lichens. Variation between the measured and modeled values may have been due to the models underestimating maximal photosynthesis and the fact that measured values were generally taken under more optimal microclimate conditions. When summed over the moss, lichen, bare wood, and bare litter + soil components of the ecosystem for the 3-month period, old-growth sub-boreal spruce forest floor lost −31.6 g C m<sup>−2</sup>.

An elevated CO<sub>2</sub> environment occurred in the moss layer at the forest floor with an average CO<sub>2</sub> level of 439 μmol mol<sup>−1</sup> in

the moss mat and 387 μmol mol<sup>−1</sup> at the moss mat surface. The moss *R. triquetrus* seemed to be CO<sub>2</sub> limited at ‘ambient’ forest floor CO<sub>2</sub> levels (430 μmol mol<sup>−1</sup>) and photosynthesis was considerably enhanced by increasing the CO<sub>2</sub> concentration to 700 μmol mol<sup>−1</sup>. In contrast, photosynthesis in the lichen *P. membranacea* was not positively influenced by increased CO<sub>2</sub> concentration. Longer term studies under more controlled microclimate conditions would be required to confirm the effect of elevated CO<sub>2</sub> on moss and lichen species in the sub-boreal spruce forest.

This study has provided increased understanding of the role of lichens and bryophytes CO<sub>2</sub> exchange at the forest floor. However, it is only a first step in gaining a better appreciation of the importance of terrestrial lichens and bryophytes to the sub-boreal spruce forest ecosystem.

## Acknowledgements

Funding for this project was provided by grant support from NSERC (ALF) and the Canadian Foundation for Climate and Atmospheric Science (ALF). We thank Dr. D. Coxson for methodological assistance, M. Jull for providing ALRF climate data, and R. Curry and D. Tainton for diligent field assistance. Dr. D. Coxson, Dr. H. Massicotte, Dr. P. Sanborn and two anonymous reviewers provided valuable comments on earlier versions of the manuscript.

## References

- Amiro, B.C., Barr, A.G., Black, T.A., Iwashita, H., Kljun, N., McCaughey, J.H., Morgenstern, K., Murayama, S., Nesic, Z., Orchansky, A.L., Saigusa, N., 2006. Carbon, energy and water fluxes at mature and disturbed forest sites, Saskatchewan, Canada. *Agric. For. Meteorol.* 136, 237–251.
- Arocena, J.M., Sanborn, P., 1999. Mineralogy and genesis of selected soils and their implications for forest management in central and northeastern British Columbia. *Can. J. Soil Sci.* 79, 571–592.
- Botting, R.S., 2005. Lichen and bryophyte diversity, nitrogen and CO<sub>2</sub> exchange from sub-boreal spruce forest floors in central British Columbia. Masters thesis. University of Northern British Columbia, Prince George, BC.
- Botting, R.S., Fredeen, A.L., 2006. Contrasting lichen, liverwort and moss diversity between old-growth and young second-growth forest on two soil texture types in central British Columbia. *Can. J. Bot.* 84, 120–132.
- Bowden, R.D., Newkirk, K.M., Rullo, G.M., 1998. Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. *Soil Biol. Biochem.* 30, 1591–1597.
- Brodo, I.M., Sharnoff, S., Sharnoff, S.D., 2001. *Lichens of North America*. Yale University Press, London.
- Canham, C.D., Coates, K.D., Bartemucci, P., Quaglia, S., 1999. Measurement and modeling of spatially explicit variation in light transmission through interior cedar-hemlock forests of British Columbia. *Can. J. For. Res.* 29, 1775–1783.
- Chazdon, R.L., Pearcy, R.W., 1991. The importance of sunflecks for forest understory plants. *BioScience* 41, 760–766.
- Coursolle, C., Margolis, H.A., Barr, A.G., Black, T.A., Amiro, B.D., McCaughey, J.H., Flanagan, L.B., Lafleur, P.M., Roulet, N.T., Morgenstern, K., Orchansky, A.L., Bernier, P.Y., Chen, J.M., Kidston, J., Saigusa, N., Hedstrom, N., 2006. Late-summer carbon fluxes from Canadian forests and peatlands along an east-west continental transect. *Can. J. For. Res.* 36, 783–800.
- Coxson, D.S., 1991. Impedance measurement of thallus moisture content in lichens. *Lichenologist* 23, 77–84.

- Coxson, D., Wilson, J.A., 2004. Carbon gain in *Cladina mitis* from mixed feather moss mats in a sub-alpine spruce-fir forest: the role of soil respiratory carbon dioxide release. *Symbiosis* 37, 307–321.
- DeLong, C., 2003. A Field Guide to Site Identification and Interpretation for the Southeast Portion of the Prince George Forest Region. British Columbia Ministry of Forests, Victoria, BC.
- DeLucia, E.H., Turnbull, M.H., Walcroft, A.S., Griffens, K.L., Tissue, D.T., Glenn, D., McSeveny, T.M., Whitehead, D., 2003. The contribution of bryophytes to the carbon exchange for a temperate rainforest. *Global Change Biol.* 9, 1158–1170.
- Dilustro, J.J., Collins, B., Duncan, L., Crawford, C., 2005. Moisture and soil texture effects on soil CO<sub>2</sub> efflux components in southeastern mixed pine forests. *For. Ecol. Manage.* 204, 85–95.
- Drewitt, G.B., Black, T.A., Nestic, Z., Humphreys, E.R., Jork, E.M., Swanson, R., Ethier, G.J., Griffs, T., Morgenstern, K., 2002. Measuring forest floor CO<sub>2</sub> fluxes in a Douglas-fir forest. *Agric. For. Meteorol.* 110, 299–317.
- Fredeen, A.L., Bois, C.H., Janzen, D.T., Sanborn, P., 2005. Comparison of coniferous forest carbon stocks between old-growth and young second-growth forests on two soil types in central British Columbia, Canada. *Can. J. For. Res.* 35, 1411–1421.
- Goulden, M.L., Crill, P.M., 1997. Automated measurements of CO<sub>2</sub> exchange at the moss surface of a black spruce forest. *Tree Physiol.* 17, 537–542.
- Green, T.G.A., Lange, O.L., 1995. Photosynthesis in poikilohydric plants: a comparison of lichens and bryophytes. In: Schulze, E.D., Caldwell, M.M. (Eds.), *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin.
- Hahn, S.C., Tenhunen, J.D., Popp, P.W., Meyer, A., Lange, O.L., 1993. Upland tundra in the foothills of the Brooks Range, Alaska: Diurnal CO<sub>2</sub> exchange patterns of characteristic lichen species. *Flora* 188, 125–143.
- Heijmans, M.M., Arp, W.J., Chapin, F.S., 2004. Carbon dioxide and water vapour exchange from understory species in boreal forest. *Agric. For. Meteorol.* 123, 135–147.
- Keeling, C.D., Whorf, T.P., 2005. Atmospheric CO<sub>2</sub> records from sites in the SIO air sampling network. In: *Trends: A Compendium of Data on Global Change, Carbon Dioxide Information Analysis Center, Oak Ridge, USA*.
- Knowles, R.D., 2004. *Peltigera*, a genus of dinitrogen fixing terricolous lichens: its influence on soil processes in the northern forests of Minnesota. PhD Thesis. University of Minnesota, Minneapolis, MN.
- Lange, O.L., Hahn, S.C., Müller, G., Meyer, A., Tenhunen, J.D., 1996. Upland tundra in the foothills of the Brooks Range, Alaska: influence of light, water content and temperature on CO<sub>2</sub> exchange of characteristic lichen species. *Flora* 191, 67–83.
- Malhi, Y., Baldocchi, D.D., Jarvis, P.G., 1999. The carbon balance of tropical, temperate and boreal forests. *Plant Cell Environ.* 22, 715–740.
- Marra, J.L., Edmonds, R.L., 1994. Coarse woody debris and forest floor respiration in an old-growth coniferous forest on the Olympic Peninsula, Washington, USA. *Can. J. For. Res.* 24, 1811–1817.
- Meidinger, D., Pojar, J., 1991. *Ecosystems of British Columbia*. British Columbia Ministry of Forests, Victoria, BC.
- Ministry of Forests, BC, Ministry of Environment BC, 1998. *Field manual for describing terrestrial ecosystems*. British Columbia Ministry of Forests and British Columbia Ministry of Environment, Lands and Parks, Victoria, BC.
- Morén, A.S., Lindroth, A., 2000. CO<sub>2</sub> exchange at the floor of a boreal forest. *Agric. For. Meteorol.* 101, 1–14.
- Murphy, B., 1996. *Prince George forest district climate normals 1951–1980*. McGregor Model Forest Association, Prince George, BC.
- Oechel, W.C., Van Cleve, K., 1986. The role of bryophytes in nutrient cycling in the taiga. In: Van Cleve, K., Chapin, F.S., Flanagan, P.W., Viereck, L.A., Dyrness, C.T. (Eds.), *Forest Ecosystems in the Alaskan taiga—A Synthesis of Structure and Function*. Springer-Verlag, New York, pp. 121–137.
- Oikos Ecological Services Ltd (OES), 1995. *Forest ecosystem/terrain mapping Aleza Lake Research Forest, Prince George Forest Region 1993–1995*. Smithers, BC.
- Palmqvist, K., 2000. Carbon economy in lichens. *Tansley Review No. 117. New Phytol.* 148, 11–36.
- Palmqvist, K., Sundberg, B., 2000. Lichen use efficiency of dry matter gain in five macro-lichens: relative impact of microclimate conditions and species-specific traits. *Plant Cell Environ.* 23, 1–14.
- Pearcy, R.W., Pflitsch, W.A., 1995. The consequences of sunflecks for photosynthesis and growth of forest understory plants. In: Schulze, E.D., Caldwell, M.M. (Eds.), *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin.
- Prentice, I.C., Farquhar, G.D., Fasham, M.J.R., Goulden, M.L., Heimann, M., Jaramillo, V.J., Khesghi, H.S., Le Quere, C., Scholes, R.J., Wallace, D.W.R., 2001. The carbon cycle and atmospheric carbon dioxide. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (Eds.), *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.
- Pypker, T.G., Fredeen, A.L., 2003. Below ground CO<sub>2</sub> efflux from cut blocks of varying ages in sub-boreal British Columbia. *For. Ecol. Manage.* 172, 249–259.
- Rayment, M.B., Jarvis, P.G., 2000. Temporal and spatial variation of soil CO<sub>2</sub> efflux in a Canadian boreal forest. *Soil Biol. Biochem.* 32, 35–45.
- Rayner, A.D., Boddy, L., 1988. *Fungal Decomposition of Wood—its Biology and Ecology*. John Wiley and Sons, Chichester.
- Russell, C.A., Voroney, R.P., 1998. Carbon dioxide efflux from the floor of a boreal aspen forest. I. Relationship to environmental variables and estimates of C respired. *Can. J. Soil Sci.* 78, 301–310.
- Schofield, W.B., 1992. *Some Common Mosses of British Columbia*. Royal British Columbia Museum, Victoria, BC.
- Sonesson, M., Gehrke, C., Tjus, M., 1992. CO<sub>2</sub> environment, microclimate and photosynthetic characteristics of the moss *Hylocomium splendens* in a subarctic habitat. *Oecologia* 92, 23–29.
- Sundberg, B., Palmqvist, K., Esseen, P.-A., Renhorn, K.-E., 1997. Growth and vitality of epiphytic lichens: II. Modelling of carbon gain using field and laboratory data. *Oecologia* 109, 10–18.
- Swanson, R.V., Flanagan, L.B., 2001. Environmental regulation of carbon dioxide exchange at the forest floor in a boreal black spruce ecosystem. *Agric. For. Meteorol.* 108, 165–181.
- Tarnawski, M.G., Green, T.G.A., Buedel, B., Meyer, A., Zellner, H., Lange, O.L., 1994. Diel changes of atmospheric CO<sub>2</sub> concentration within, and above, cryptogam stands in a New Zealand temperate rainforest. *New Zealand J. Bot.* 32, 329–336.
- Van Der Heijden, E., Verbeek, S.K., Kuiper, P.J.C., 2000. Elevated atmospheric CO<sub>2</sub> and increased nitrogen deposition: effects on C and N metabolism and growth of the peat moss *Sphagnum recurvum* P. Beauv. var. *mucronatum* (Russ.). *Warnst. Global Change Biol.* 6, 201–212.
- Vourlitis, G.L., Oechel, W.C., Hastings, S.J., Jenkins, M.A., 1993. A system for measuring *in situ* CO<sub>2</sub> and CH<sub>4</sub> flux in unmanaged ecosystems: an arctic example. *Funct. Ecol.* 7, 369–379.
- Watson, R., Noble, I.R., Bolin, B., Ravindranath, N.H., Verardo, D.J., Dokken, D.J., 2000. *IPCC Special Report. Summary for Policymakers: Land Use, Land-Use Change, and Forestry. IPCC Plenary XVI, Montreal, Canada*.
- Whitehead, D., Gower, S.T., 2001. Photosynthesis and light-use efficiency by plants in a Canadian boreal forest ecosystem. *Tree Physiol.* 21, 925–929.