

HOKKAIDO UNIVERSITY

Title	Variations in biomass, production, and respiration of fine roots in a young larch forest
Author(s)	崔, 鋭
Citation	北海道大学. 博士(農学) 甲第14823号
Issue Date	2022-03-24
DOI	10.14943/doctoral.k14823
Doc URL	http://hdl.handle.net/2115/88855
Туре	theses (doctoral)
File Information	Cui_Rui.pdf



## Variations in biomass, production, and respiration of fine roots in a young larch forest

(カラマツ若齢林における細根のバイオマス,生産量および 呼吸の変動)

# Hokkaido University Graduate School of Agriculture Frontiers in Environment Sciences Doctor Course

Cui Rui

## Abstract

Soil respiration ( $R_s$ ) accounts for 30–80% of total respiration in forest ecosystems, indicating  $R_s$  plays a crucial role in terrestrial carbon cycles. The  $R_s$  is composed of root respiration ( $R_r$ ) and microbial heterotrophic respiration ( $R_h$ ). Plant roots are different in metabolism and functions according to size and order. Fine roots (typically < 2 mm in diameter) perform important functions and govern belowground carbon cycles. However, the phenological variation of the functions is not well understood. Thus, we adopted an approach to partition  $R_r$  into respirations for growth ( $R_g$ ), maintenance ( $R_m$ ), and ion uptake ( $R_{ion}$ ) using modified empirical models. We conducted field experiments on  $R_s$  and fine root dynamics, and transpiration in a larch-dominated young forest on the bare ground after removing surface organic soil to parameterize the models.

The field experiments were conducted in 2017–2018 (E1) and 2019–2020 (E2) in a regenerating forest dominated by Japanese larch in Tomakomai, Hokkaido. The top organic soil was removed after typhoon disturbance in 2004. Collar pairs consisting of control (CC) and trenched (TC) ones were installed at 0.5 m (N) and 1 m (F) from isolated larch trees (n = 10 (E1) and 18 (E2)). Soil CO<sub>2</sub> fluxes ( $R_{CC}$  and  $R_{TC}$ ) were periodically measured on the collars by a chamber method. The  $R_{CC}$  and  $R_{TC}$  were continuously estimated throughout the experimental periods from soil temperature ( $T_s$ ) using exponential equations. CO<sub>2</sub> efflux through dead root decomposition ( $R_{DR}$ ) in trenched collars was also estimated. The  $R_h$  was calculated as  $R_{TC} - R_{DR}$ , and  $R_r$  was derived as  $R_{CC} - R_h$ . Fine root biomass ( $B_f$ , g DM m<sup>-2</sup>) and production ( $P_f$ , g DM m<sup>-2</sup> d<sup>-1</sup>) were periodically measured in CC by the sequential coring and ingrowth core methods, respectively. In addition, sap flow was measured by a thermal dispersion method only in E2. The following two models were applied to partition  $R_r$  (g C m<sup>-2</sup> d<sup>-1</sup>):

$$R_r = R_m + R_g = c_1 \cdot P_f + d_1 \cdot exp(f_1 \cdot T_s) \cdot (B_f + B_c) \quad (1)$$
$$R_r = R_m + R_g + R_{ion} = c_2 \cdot P_f + d_2 \cdot exp(f_2 \cdot T_s) \cdot (B_f + B_c) + g \cdot T_r \cdot B_f \quad (2)$$

where *c*, *d*, *f*, and *g* are fitting parameters,  $B_c$  coarse root biomass (g DM m<sup>-2</sup>), and  $T_r$  transpiration per fine root biomass (g H<sub>2</sub>O g DM<sup>-1</sup> d<sup>-1</sup>).

Annual  $R_s$  was 493 ± 45 (N) and 311 ± 34 g C m<sup>-2</sup> yr<sup>-1</sup> (F) (mean ± standard error) in E1, and  $R_r$  accounted for 37% (N) and 16% (F) of  $R_s$ . Despite no seasonal variation in  $B_f$ ,  $P_f$ decreased in the cold season. Annual  $P_f$  was 81 ± 22 (N) and 41 ± 10 g DM m<sup>-2</sup> yr<sup>-1</sup> (F), and annual mean  $B_f$  was 70 ± 9 (N) and 13 ± 3 g DM m<sup>-2</sup> yr<sup>-1</sup> (F). Model 1 (M1) was significantly parameterized ( $r^2 = 0.59$ , p < 0.001) using the field data (n = 50). Annual  $R_r$  was estimated to be 107 g C m<sup>-2</sup> yr<sup>-1</sup> and accounted for 25% of  $R_s$ . The  $R_r$  was partitioned into fine root  $R_g$ , fine root  $R_m$ , and coarse root  $R_m$  by 30, 44 and 26%, respectively. In E2, annual  $R_s$  was  $610 \pm 26$  (N) and 474 ± 52 g C m<sup>-2</sup> yr<sup>-1</sup> (F), and  $R_r$  accounted for 47% and 45% of  $R_s$ , respectively. The  $B_f$ increased slightly in the growing season, whereas  $P_f$  clearly decreased in the cold season and peaked in July. Annual  $P_f$  was  $115 \pm 7$  (N) and  $102 \pm 10$  g DM m<sup>-2</sup> yr<sup>-1</sup> (F), and mean  $B_f$  was  $133 \pm 13$  (N) and  $78 \pm 14$  g DM m<sup>-2</sup> yr<sup>-1</sup> (F). The  $B_c$  was less than a third of  $B_f$ . Models 1 and 2 were significantly parameterized ( $r^2 = 0.51-0.53$ , p < 0.001) using the field data (n = 144). Although the parameters of d ( $R_m$  at 0°) and f (the temperature coefficient of  $R_m$ ) were almost the same between the two models, the parameter of  $R_g$  (c) was smaller for M1. The  $R_m$  and  $R_{ion}$ peaked in June–July, whereas  $R_g$  peaked earlier in June. Annual  $R_r$  was estimated to be 215 g C m<sup>-2</sup> yr<sup>-1</sup> and accounted for 41% of  $R_s$ . The  $R_r$  was partitioned into fine root  $R_g$ , fine root  $R_m$ , coarse root  $R_m$ , and fine root  $R_{ion}$  by 32, 46, 13 and 9%, respectively.

The two models were significantly fitted to field data. Although all parameters in M1 were significant determined in both experiments, those from E2 would be more robust because of more data sets for curve fitting and the addition of spatial distribution of coarse root biomass. The parameters of *d* and *f* related to  $R_m$  were almost the same between M1 and M2 in E2; the  $Q_{10}$  of  $R_m$  calculated from parameter *f* was 2.46–2.61. However, the parameter of *c* for  $R_g$  was lower by 20% in M2, suggesting that  $R_{ion}$  was assigned to  $R_g$  in M1, because  $R_{ion}$  was reported to be proportional to  $R_g$  from laboratory experiments. Using M2, we estimated that fine roots account for 87% of total  $R_r$  annually, and fine root  $R_r$  was partitioned into  $R_g$ ,  $R_m$ , and  $R_{ion}$  by 37, 53, and 10%, respectively. The  $R_g$ ,  $R_m$ , and  $R_{ion}$  varied according to the seasonal variations of  $P_f$ ,  $T_s$ , and  $T_r$ , respectively.

To partition  $R_r$  into  $R_g$ ,  $R_m$ , and  $R_{ion}$ , we applied modified empirical models and parameterized them using seasonal field data of soil CO<sub>2</sub> efflux,  $B_f$ ,  $P_f$ ,  $B_c$ ,  $T_s$ , and  $T_r$  measured in a young larch-dominated forest regrowing after the removal of surface organic soil. In such a simplified filed condition, we succeeded in significant partitioning of root respiration in a filed condition. Despite ignoring coarse root growth, the results suggest that our approach is capable of partitioning root respiration.

## Contents

A	bstract	i
1.	Introduction	.1
	1.1 Background	.1
	1.2 Objectives	.3
2.	Material and method	.4
	2.1 Study site	.4
	2.2 Experimental design	.8
	2.2.1 Experiment 1 (2017–2018)	.8
	2.2.2 Experiment 2 (2019–2020)	.8
	2.3 Soil CO <sub>2</sub> efflux	10
	2.4. Decomposition of dead roots	12
	2.5. Biomass and production of fine roots	14
	2.6. Sap flow	16
	2.7. Partitioning root respiration	17
	2.8. Statistical analysis	19
3.	Results	20
	3.1 Experiment 1 (2017–2018)	20
	3.1.1 Environmental conditions	20
	3.1.2. Soil CO <sub>2</sub> efflux	22
	3.1.3. Biomass and production of fine roots	26
	3.1.4. Partitioning root respiration	29
	3.2 Experiment 2 (2019–2020)	32
	3.2.1 Environmental conditions	32
	3.2.2. Soil CO <sub>2</sub> efflux	33
	3.2.3. Biomass and production of fine roots	37
	3.2.4. Sap flow	40
	3.2.5. Partitioning root respiration	41

4. Discussion	
5. Conclusions	48
Acknowledgement	49
References	

## 1. Introduction

## 1.1 Background

Net ecosystem production (NEP) is the tiny balance of two huge CO<sub>2</sub> fluxes of gross primary production (GPP) and ecosystem respiration (RE). Soil respiration ( $R_s$ ) accounts for 30–80% of RE on an annual basis in forest ecosystems (Davidson et al. 2006) but typically 70% in temperate forests (Ryan and Law 2005). On a global scale,  $R_s$  (98 Pg C yr<sup>-1</sup>; (Bond-Lamberty and Thomson 2010)) accounts for about 80% of GPP (123 Pg C yr<sup>-1</sup>; (Beer et al. 2010)). These facts indicate that  $R_s$  plays a crucial role in terrestrial carbon cycles.

The  $R_s$  is composed of autotrophic respiration ( $R_a$ ) and heterotrophic respiration ( $R_h$ ). The  $R_a$  is equivalent to respiration from roots (root respiration:  $R_r$ ) using photosynthate and nonstructural carbohydrates as substrate, whereas  $R_h$  is the microbial decomposition of soil organic matter (SOM) and carbohydrates (NSC) derived from roots (Moyano et al. 2009). The  $R_a$  and  $R_h$  respond to temperature and moisture in a different manner (Scott-Denton, Rosenstiel, and Monson 2006; Lavigne, Foster, and Goodine 2004; Boone et al. 1998). Thus, to examine the environmental response of  $R_s$ ,  $R_s$  has been partitioned into  $R_r$  and  $R_h$  by root-exclusion methods, such as trenching (Epron 2009). In forest ecosystems, the raio of  $R_r$  and  $R_s$  ( $R_r / R_s$ ) ranged between 10 and 90% depending on vegetation types and seasons (Hanson et al. 2000) but typically 45–50% (Subke, Inglima, and Francesca Cotrufo 2006).

Roots are classified into non-woody fine roots and woody coarse roots by anatomical traits and physiological functions. Fine roots are physiologically active apexes of root branches and conventionally defined as thinner roots < 2 mm in diameter (*e.g.* Brunner *et al.*, 2013; Finér *et al.*, 2011). Fine roots absorb and transport water and nutrients, whereas coarse roots, which are  $\geq 2$  mm in diameter, transport them and anchor and support their aboveground part. The net primary production (NPP) of fine roots is large and consequently accounted for 22% of global terrestrial NPP (McCormack *et al.*, 2015). In boreal forests, although fine root biomass accounted for only 16% of root biomass, 73% of all belowground NPP arose from fine root NPP (Yuan and Chen, 2010); fine root NPP can be an index of belowground NPP (Yuan and Chen, 2013). Because root turnover rates become faster as root diameter becomes thinner (Yuan and Chen, 2010), fine roots supply a large amount of litter to the soil together with labile exudate, which stimulate soil microbial activities and change soil carbon stock. Majority of soil carbon in forest ecosystems certainly results from fine root turnover (Richter *et al.*, 1999). The respiration rate normalized by dry matter was much higher from fine roots than from coarse roots (Makita *et al.* 2012; Rewald *et al.*, 2014). When the respiration rate was scaled up based on biomass data, stand-level respiration of fine roots accounted for 50–70% of  $R_s$  in plantations of acacia and eucalyptus (Chen *et al.*, 2009; Marsden *et al.*, 2008). Thus, fine roots are the most important component contributing to belowground carbon dynamics in forest ecosystems (Finér *et al.*, 2011). However, the function of fine roots in carbon cycles is not well understood yet (McCormack *et al.*, 2015), because the production and turnover of fine roots substantially depend on plant functional types and climates (Lambers *et al.*, 2008; Yuan and Chen, 2012). In addition, information is still lacking on belowground phenology (Abramoff and Finzi, 2015).

Fine root respiration has been frequently partitioned into growth ( $R_g$ ) and maintenance ( $R_m$ ) components (Amthor, 2000; McCree, 1974; Thornley, 1970; Penning de Vries, 1974), plus occasionally into ion uptake ( $R_i$ ) (Chapin *et al.*, 2011; Johnson, 1990; Lambers *et al.*, 2008) to examine belowground phenology and predict root respiration using a simple model, in which  $R_g$ ,  $R_m$  and  $R_i$  are proportioned to growth (production), biomass and nutrient uptake of fine roots, respectively. Terrestrial biosphere models, such as Biome-BGC, are adopting the partitioning model (Sun *et al.*, 2017). Although there is a criticism that the scientific basis is lacking in the quantitative division between  $R_g$  and  $R_m$  (Cannell and Thornley, 2000; Sweetlove *et al.*, 2013), experimental data indicate that the model is a valuable tool in understanding the carbon balance of plants and the ecological controls on plant respiration (Lambers *et al.*, 2008; Chapin *et al.*, 2011). The model has been parameterized and verified mostly based on laboratory experiment data taken in the controlled environment (*e.g.* Lambers *et al.*, 2008; Thongo M'Bou *et al.* 2010). Only two studies from field experiments reported the partitioning of fine root respiration using the model (George *et al.*, 2003; Sun *et al.*, 2020). George *et al.* (2003) estimated annual values of  $R_g$ ,  $R_m$  and  $R_i$  (due to nitrogen uptake) from a short-term chamber experiment and literature

information. Sun *et al.* (2020) conducted a field experiment for more than a year in adjacent two mature forests: an evergreen spruce plantation and a larch-dominated deciduous forest, which are located next to our study site. They quantified annual  $R_g$  and  $R_m$  and discussed the difference between the two forests. However, the results can have large uncertainty arising from field conditions, such as a high ratio of coarse roots, rich understory species in the deciduous forest and a high spatial variation in litter accumulation, root density and SOM especially in the deciduous forest, because the forests were mature at the age over 35 years and the deciduous forest was damaged by windthrow. These site conditions probably increased the spatial variation of  $R_h$  and the contamination from other roots than fine roots, which resulted in uncertainty in  $R_r$  separation from  $R_s$  and model parameterization. Although it is a challenge to substantially decrease uncertainty in field experiments, the following site conditions can improve data reliability: 1) homogeneity in tree age, 2) young forest with less litter accumulation and coarse roots, 3) no or little understory species, and 4) a small amount of SOM.

## **1.2 Objectives**

We measured seasonal variations in soil CO<sub>2</sub> efflux and fine root dynamics from 2017 to 2018 and 2019 to 2020 in a young larch-dominated forest, respectively, in two experiments. The forest was regenerating on the topsoil-removed bare ground to meet the above conditions. Using carefully measured field data, the objectives of this study are 1) to continuously separate fine root  $R_r$  from total  $R_s$ , 2) to robustly parameterize the partitioning model, 3) to partition the  $R_r$  into  $R_g$ ,  $R_m$  and  $R_{ion}$  using a modified model, and 3) to show the phenological variations of fine root respiration along with fine root production and biomass.

## 2. Material and method

## 2.1 Study site

The field experiments were conducted twice in 2017–2018 (Experiment 1) and 2019–2020 (Experiment 2) in a regenerating young forest dominated by Japanese larch (Larix kaempferi) with sparse Japanese white birth (Betula platyphylla) in Tomakomai national forest, southern Hokkaido, Japan (42°44.27'N, 141°31.42'E, 116 m above sea level by Google Earth). The study site was used to be a larch plantation (Photo 2.1) established on volcanogenous regosol with a high-water permeability but was severely destroyed by a windstorm due to a typhoon in 2004 (Photo 2.2) (Sano et al., 2010); all stems of both fallen and standing trees were removed (Phot 2.3), and then organic surface soil (A horizon) was scraped out together with stumps, coarse woody debris, litter accumulation, regenerating understory species and buried seeds in 2006 (Photo 2.4). Because the B horizon is lacking, unweathered pumice stones (C horizon) have been exposed. Wind-blown seeds of larch and birch first germinated on the C horizon in a horizontal flat place in 2007 and have been growing despite poor nutrients (Photo 2.5). The site was located between two forests used for field experiments by Sun et al. (2020). The ground surface was sparsely covered with stunted ground vegetation dominated by red raspberry (Rubus idaeus). Aboveground biomass (AGB) of trees taller than 2 m was 16.7, 20.9, 27.3 and 32.8 t ha<sup>-1</sup>, respectively, from 2017 to 2020 (Fig. 2.1), of which larch trees accounted for 81-87% (Photo 2.6). Tree density, tree height and diameter at 1.3 m height in 2018 were 3380 trees  $ha^{-1}$ , 4.27 ± 1.21 m and 3.9 ± 2.2 cm (mean ± 1 standard deviation (SD)), respectively. In 2016, bulk density, total carbon (C) concentration, total nitrogen (N) concentration and C/N ratio were  $0.446 \pm 0.042$  g cm- 3,  $15.2 \pm 13.8$  g kg<sup>-1</sup>,  $0.742 \pm 0.794$  g kg<sup>-1</sup> and  $24.5 \pm 5.8$  (mean  $\pm 1$ standard deviation (SD)), respectively, for top 15-cm-thick fine soil (< 2 mm). The C concentration showed a decreasing tendency with a distance from tree stems according to the amount of leaf litter fall; the C concentration accounted for about 30% of that of a nearby mature larch-dominated forest studied by Sun et al. (2020).

Decadal mean annual air temperature and precipitation were  $8.1 \pm 0.3$  °C and  $1305 \pm 202$  mm yr<sup>-1</sup>, respectively, from 2011 to 2020 at a meteorological station (Tomakomai) 14 km

apart from the study site. The highest and lowest monthly mean air temperatures in the same decade were 21.0°C in August and -3.9°C in January, respectively. Snow usually covers the ground for about four months between early December and early April.



**Fig. 2.1** Interannual variation in forest total biomass, larch biomass and larch root biomass from 2015 to 2020.





Photo 2.1 The larch forest before typhoon disturbance

Photo 2.2 The study site just after typhoon disturbance (September 2004)



Photo 2.3 The study site after the removal of fallen trees (May 2005).



Photo 2.4 The study site after the surface soil removal (May 2007).



Photo 2.5 Regrowing visitation (June 2010).



Photo 2.6 Larch-dominated young forest (June 2017).

## 2.2 Experimental design

## 2.2.1 Experiment 1 (2017–2018)

Ten pairs of aluminum collars were installed in May 2017 around isolated larch trees. Six pairs of collars were set at 0.5 m (Near), and the others were set at 1.0 m (Far) from the target larch stems (Photo 2.7). The other trees were more than 1.5 m away from collars. In each pair, two collars were set at a 0.4 m space on the bare ground without ground vegetation. Collars were 0.5 m  $\times$  0.5 m in size and inserted 3 cm deep into the soil. We trenched a collar (TC) of each pair in early July 2017 by inserting four PVC boards 20 cm deep into the soil around the collar to exclude root respiration. We found by eye from soil profiles that almost all roots appeared in the top 15 cm layer. The other collar (CC) was used for fine root sampling without trenching.

## 2.2.2 Experiment 2 (2019–2020)

All collars in Experiment 1 were relocated in July 2019. Nine pairs of two collars with a 0.4 m space were installed at 0.5 m (Near) and at 1.0 m (Far) from the target larch stems, respectively. Similarly with Experiment 1, collars were  $0.5 \text{ m} \times 0.5 \text{ m}$  in size and inserted 3 cm deep into the soil. Of each pair, one collar was trenched (TC) and another was for control (CC).



**Photo 2.7** Collars were set at 0.5 m (Near), and the others were set at 1.0 m (Far) from the target larch stems.



Photo 2.8 Collars were set as control (CC) (right) and trench (TC) (left) as a pair.

#### 2.3 Soil CO<sub>2</sub> efflux

Experiment 1 was conducted at intervals of about three weeks between May 2017 and August 2018 with a six-month suspension from mid-November 2017 to mid-May 2018 mainly because of snow. Experiment 2 was conducted with the same interval from August 2019 to November 2020 and suspended during the snow season between mid-November 2019 to mid-April 2020. Soil CO<sub>2</sub> efflux was manually measured on each collar with the same method as in (Sun et al. 2020; Sun, Teramoto, et al. 2017). Young plants germinating in collars were carefully pulled out before measurement, though it was rare. The measurement was conducted between 10:00 and 16:00 on each date with a closed-chamber system equipped with two 0.5-m-tall cubic chambers, an air pump, a datalogger (CR1000; Campbell Scientific Inc., Logan, UT, USA) and a CO<sub>2</sub> analyzer (LI820; Li-Cor Inc., Lincoln, NB, USA) (Photo 2.9). The datalogger was used to control the system and record data. CO<sub>2</sub> concentration in the chamber was measured every 5 s during chamber closing for 3 min. The CO<sub>2</sub> increasing rate was determined from the continuous data by the least squares method, and then soil CO<sub>2</sub> efflux was calculated from the increasing rate and chamber air temperature using the following equation:

$$R = \frac{VP}{RST} \frac{dC}{dt} \tag{1}$$

where *V* is the chamber volume (m<sup>3</sup>), *S* is the soil surface area inside the chamber (m<sup>2</sup>), *P* is the air pressure (Pa), *T* is the air temperature inside the chamber (K), dC/dt is the changing rate of CO<sub>2</sub> concentration (µmol mol<sup>-1</sup> s<sup>-1</sup>) determined using the least-square method, and the *R* is the gas constant (8.314 Pa m3 K<sup>-1</sup> mol<sup>-1</sup>). Soil temperature (*T*<sub>s</sub>) at a depth of 5 cm and volumetric soil water content (SWC) of the top 5-cm soil were measured immediately after the efflux measurement in each collar by a thermometers (MHP; Omega Engineering, Stanford, CA, USA) (Photo 2.8) and a soil moisture sensor (SM150; Delta-T Device Ltd., Cambridge, UK). Also, *T*<sub>s</sub> at a depth of 6 cm and SWC at a depth of 3 cm were monitored at four points at a nearby station 150 m from the study site (Hirano, Suzuki, and Hirata 2017); we used the data measured at an open place where C horizon was exposed like the study site.

After trenching in early July 2017 and lately July 2019, CO<sub>2</sub> efflux from TC ( $R_{TC}$ )

corresponded to the sum of original  $R_h$  and CO<sub>2</sub> emissions through the decomposition of dead roots ( $R_{DR}$ ) by trenching (Epron 2009). The method to estimate  $R_{DR}$  is described later. Meanwhile, CO<sub>2</sub> efflux from CC ( $R_{CC}$ ) corresponded to total  $R_s$ . Although CC was disturbed by soil sampling, its effect on CO<sub>2</sub> efflux was insignificant because sampling area was negligible (Sun et al. 2020). Thus, the sum of fine and coarse root respirations ( $R_r$ ) was determined for each pair of collars, as  $R_r = R_{CC} - R_{TC} - R_{DR}$ . Since the distance from a larch tree was the same, two collars in a pair were expected to have similar root density and litter fall.

We applied the following simple exponential model to analyze the effect of  $T_s$  (°C) on soil CO<sub>2</sub> efflux (*R*c, µmol m<sup>-2</sup> s<sup>-1</sup>) on each collar.

$$Rc = a \cdot exp(b \cdot T_s) \qquad (2)$$

where *a* and *b* are fitting parameters. From the temperature response parameter (*b*), we calculated  $Q_{10}$ , which is the factor by which  $R_c$  increases with a 10°C temperature rise. In addition, the effect of SWC was analyzed using the temperature-normalized  $R_c$  at a base temperature ( $T_b$ ).

$$R_b = R_c \cdot exp(b \cdot (T_b - T_s)) \tag{3}$$

In this study  $T_b$  was set at 15°C, mean  $T_s$  for the snow-free season. Using the relationship of  $R_c$  with  $T_s$  and/or SWC,  $R_{CC}$  and  $R_{TC}$  were calculated half hourly on every collar from the monitoring data of  $T_s$  and/or SWC. Daily  $R_r$  was calculated for each pair from daily  $R_{SC}$ ,  $R_{TC}$  and  $R_{DR}$ .



Photo 2.8 The chamber system.

## 2.4. Decomposition of dead roots

We conducted a root litter bag experiment to determine the decay constant (*k*) (Gholz *et al.*, 2000; Silver and Miya, 2001; Sun *et al.* 2020). In June 2017, we collected topsoil to a depth of 15 cm from the total area of 1 m<sup>2</sup> and extracted fine toots from mixed soil samples with the same method as described later. The root samples were air-dried and weighed, and then some were oven dried. We determined the water content of air-dried samples from the dry weight and calculated the dry weight of air-dried samples. Air-dried samples corresponding to 0.48 g of dry weight were put into each 2-mm mesh bag of 10 cm × 10 cm in size. The sample amount was equivalent to measured root density. A total of 60 bags were buried at a depth of 10 cm at about 1 m from a larch stem in July 2017 (Photo 2.9). Ten bags were collected six times until October 2018, and remaining samples were oven-dried and weighed. The *k* was determined by fitting the following equation (Wieder and Lang, 1982) to the data.

 $Y = Y_0 \cdot exp(-k \cdot t) \tag{4}$ 

where *Y* is the dry weight of remaining root samples in bags (g bag<sup>-1</sup>) at elapsed time *t* (days), *Y*<sub>0</sub> the initial dry weight of root samples (0.48 g bag<sup>-1</sup>) and *k* the decay constant (d<sup>-1</sup>). As for the *k* of coarse roots, we used a reported value of  $4.4 \times 10^{-4} d^{-1} (= 0.16 \text{ yr}^{-1})$  from a nearby larch-dominated forest (Sun et al. 2020).

The  $R_{DR}$  (g C m<sup>-2</sup> d<sup>-1</sup>) at elapsed time *t* (days) was calculated daily, separately for fine and coarse roots using the following equation.

$$R_{DR} = C_c \cdot (X_{t-1} - X_t) = c \cdot X_0 \cdot \exp(-k \cdot t) \cdot \{\exp(k) - 1\}$$
(5)

where  $C_c$  is the carbon concentration of roots (g g<sup>-1</sup>),  $X_t$  the dry weight of remaining dead roots (g m<sup>-2</sup>) at *t* and  $X_0$  the initial dry weight of dead roots (g m<sup>-2</sup>). For both fine and coarse roots, we used  $C_c$  of 0.484 g g<sup>-1</sup> (Neumann *et al.*, 2020), which was the average value of fine roots in European forests. The  $X_0$  of fine roots were set at 66 (Near) and 29 (Far) g m<sup>-2</sup>, which were based on fine root biomass measured by soil core sampling from CC in July 2017. As for coarse roots,  $X_0$  were determined to be 55 (Near) and 49 (Far) g m<sup>-2</sup> from soil sampling down to 15 cm in May and September 2019.



Photo 2.9 Litter bags were burned in July 2017.

#### 2.5. Biomass and production of fine roots

The biomass and production of fine roots were measured with the same method as in Sun *et al.* (2020). Fine root biomass density ( $B_f$ , g m<sup>-2</sup>) was measured by soil coring five times from July 2017 to August 2018 for about 14 months with a six-month suspension from mid-November 2017 to mid-May 2018 in Experiment 1. In Experiment 2, the measurement was conducted from September 2019 to November 2020 with the snow season suspension. Soil cores were extracted down to 15 cm with a stainless-steel edged tube with an inner diameter of 2.4 cm (Photo 10). We sampled three cores from each CC every time, using perforated board for positioning. Sampling positions were randomly selected from a grid with 8 cm spacing and not reused. In total, the pit area by sampling amounted to 68 cm<sup>2</sup> for each CC, which accounted for only 2.7% of each collar area. Soil samples were put into PVC tubes and stored in a freezer, and then dispersed in a vat filled with tap water. Only living fine roots with a diameter < 2 mm were visually extracted, dried at 70°C for 48 hours and weighed. The fine roots should have originated from larch trees because the other plants did not exist in the vicinity.

Fine root production rates ( $P_{\rm f}$ , g m<sup>-2</sup> period<sup>-1</sup>) were measured by the ingrowth core method (*e.g.* Vogt *et al.*, 1998) with plastic hair curlers wrapped in a 2 mm mesh sheet. The cores were 20 cm long and had a diameter of 2.3 cm. Air-dried fine soil, which was collected from the study site and sieved through 2 mm meshes, was put into the cores up to 15 cm. For each CC, three cores were inserted down to 15 cm into the pits made by soil core sampling (Photo 11), and three cores inserted previously were collected simultaneously with the core sampling. Collected ingrowth cores were put into PVC tubes and stored in a freezer, and then dry weight of fine roots was determined with the same method as described above. Fine roots penetrate root-free soil in ingrowth cores through meshes during a period between two sampling dates. Thus, the dry weight of fine roots in ingrowth cores corresponds to  $P_{\rm f}$ . Also, annual fine root mortality ( $M_{\rm f}$ , g m<sup>-2</sup> yr<sup>-1</sup>) was calculated as a difference between annual  $P_{\rm f}$  and an annual difference in  $B_{\rm f}$  ( $\Delta B_{\rm f}$ ) ( $M_{\rm f} = P_{\rm f} - \Delta B_{\rm f}$ ). We converted dry weight to carbon using a factor of 0.484. In addition, turnover rates were calculated as the ratio of annual  $P_{\rm f}$  and mean  $B_{\rm f}$  (Brunner *et al.*, 2013).



Photo 2.10 Sequential soil core sampling method.



Photo 2.11 Ingrowth core method.

#### 2.6. Sap flow

Sap flow velocity was measured for three trees of a normal size by the thermal dispersion method (Granier, 1987) only in Experiment 2 form July 2019 to November 2020 with a suspension during the leafless season between mid-November 2019 and mid-April 2020 (Table 2.1). Sap flow sensors (CUP-SPF-M, Climatec Inc., Tokyo, Japan) were installed at a height 25 cm below the lowest branch to measure whole transpiration of each tree (Photo 2.12). From a destructive sampling of some trees in the study site, we presumed that the stem area (25–72 cm<sup>2</sup>) at the sensor heigh was occupies by sap area. Thus, sap flow rates were calculated as the product of sap flow velocity and the stem area. Although sap flow rates, transpiration rates and water uptake rates by roots are not the same exactly, they are almost the same on a daily basis.

Because diameters at breast height (DBH) were  $54.4 \pm 28.3$  and  $58.2 \pm 30.4$  cm (mean  $\pm 1$  SD; n = 215-225) for larch trees in the study site in 2019 and 2020, respectively, the three trees were almost within the range of mean  $\pm 1$  SD in DBH. Coarse root biomass was estimated from DBH using an allometric equation for each tree (Table 2.1), and fine root biomass (diameter < 2 mm) was estimated from the coarse root biomass using a ratio between fine and coarse root biomass of 0.185, which was determined for boreal trees (Yuan and Chen, 2010). Daily sap flow rates were normalized by the fine root biomass ( $T_r$ , g H<sub>2</sub>O g dry matter (DM)<sup>-1</sup> d<sup>-1</sup>) to estimate daily water uptake rates by fine roots in each CC.

	DBH		Total bi	Total biomass		Coarse root biomass		Fine root biomass	
Tree	(mm)		(kg D	(kg DM)		(kg DM)		(kg DM)	
	2019	2020	2019	2020	2019	2020	2019	2020	
531	36.9	42.5	5.9	7.8	1.02	1.34	0.19	0.25	
A514	63.9	71.3	16.2	20.3	2.73	3.38	0.50	0.63	
A513	88.9	94.8	25.9	31.9	4.28	5.22	0.79	0.97	

Table 2.1 Specifics of sample trees, in which sap flow sensors were installed.



Photo 2.12 Sap flow sensor installation

## 2.7. Partitioning root respiration

Originally,  $R_r$  was partitioned into  $R_g$  and  $R_m$  by the following equation (Amthor 2000; Thornley 1970).

$$R_r = R_g + R_m = g_R \cdot P_f + m_R \cdot B_f \tag{6}$$

where  $g_R$  is the growth respiration coefficient and  $m_R$  the maintenance respiration coefficient. We modified the equation as follows, considering field conditions.

$$R_r = R_g + R_m = c \cdot P_f + d \cdot exp(f \cdot T_s) \cdot (B_f + B_c) : \text{ Model } 1$$
(7)

$$R_r = R_g + R_m + R_{ion} = c \cdot P_f + d \cdot exp(f \cdot T_s) \cdot (B_f + B_c) + g \cdot T_r \cdot B_f: \text{ Model 2 (8)}$$

where *c* corresponds to  $g_R$  (g C g DM<sup>-1</sup>), *d* the  $R_m$  of unit biomass at 0°C (g C g DM<sup>-1</sup> d<sup>-1</sup>), *f* a temperature coefficient (°C<sup>-1</sup>),  $B_c$  coarse root biomass (g m<sup>-2</sup>), *g* a transpiration coefficient (g C g H<sub>2</sub>O<sup>-1</sup>), *T<sub>r</sub>* daily sap flow rate per unit fine root biomass (g H<sub>2</sub>O g DM<sup>-1</sup> d<sup>-1</sup>). In Model 2, respiration for ion uptake ( $R_{ion}$ , g C m<sup>-2</sup> d<sup>-1</sup>) was introduced. We added temperature effect on  $R_m$  in an exponential form, because temperature directly affect  $R_m$  (Moyano et al. 2009; Thornley 2011). Although tree survey suggested that coarse roots had grown during the study period, we assumed that the contribution of coarse roots to  $R_g$  was negligible because of no available data. However, we incorporated  $B_c$  into the second term on the assumption that coarse roots showed the same temperature response as fine roots. We assumed that  $R_{ion}$  is proportional to water uptake in the condition of low root density, such as in our study site (Oyewole et al., 2014; Henriksson et al., 2021).

We determined the parameters of *c*, *d*, *f* and *g* by curve fitting to measured data of  $R_r$ ,  $P_f$ ,  $T_s$ ,  $B_f$ ,  $B_c$  and  $T_r$ . For the fitting, mean daily  $R_r$  (g C m<sup>-2</sup> d<sup>-1</sup>) for each period of core sampling intervals was used. The  $P_f$  was converted to daily value (g m<sup>-2</sup> d<sup>-1</sup>). The  $B_f$  (g m<sup>-2</sup>) was a mean of two consecutive data measured at the beginning and the end of the period. The  $T_s$  (°C) was mean soil temperature for the period. The  $B_c$  were set at 55 and 49 g m<sup>-2</sup> at Near and Far in Experiment 1, and  $B_c$  measured in each CC were used in Experiment 2, neglecting its seasonal variation. All the data were prepared from each pair of collars (n = 10 (Experiment 1) and 18 (Experiment 2)) for each period (n = 5 (Experiment 1) and 8 (Experiment 2)). Thus, the data size for curve fitting was 50 and 144 in Experiment 1 and Experiment 2, respectively. In comparison with the previous study (Sun et al. 2020), the data size for curve fitting increased considerably from five to 50 or 144 to obtain a robust result. In addition, we incorporated  $B_c$  in the second term, considering the temperature response of  $R_m$  of coarse roots and herbaceous roots. In addition, we added  $R_{ion}$  in Model 2 for Experiment 2.

## 2.8. Statistical analysis

We applied Student's *t*-test to compare two means, assuming homoscedasticity. Also, we applied two-way repeated measures ANOVA to test the effects of factors. The significance of curve fitting and parameters were tested using *F*-test and *t*-test, respectively. All the statistical analyses were conducted with a software package (Origin Pro 2015 J; Origin Lab Corporation, Northampton, MA, USA).

## 3. Results

## 3.1 Experiment 1 (2017–2018)

## **3.1.1 Environmental conditions**

We conducted a field experiment on fine root dynamics from July 2017 to August 2018. During the annual period between August 2017 and July 2018, mean air temperature and total precipitation were 7.7°C and 1425 mm at the Tomakomai meteorological station. The annual mean air temperature was low beyond the range of its decadal mean  $\pm 1$  SD (8.1  $\pm$  0.3°C) between 2010 and 2019, whereas annual precipitation was within the decadal range (1346  $\pm$  159 mm yr<sup>-1</sup>). The  $T_s$  at a depth of 6 cm showed an obvious seasonal variation with the maximum of 27.4°C in mid-July 2017 and the minimum of -0.64°C in early December 2017 on a daily basis (Fig. 1a). Under snow cover,  $T_s$  was stable at -0.26 0.14°C between December and February. The SWC at 3 cm increased to 0.21 m<sup>3</sup> m<sup>-3</sup> in late November and rapidly decreased by soil freezing in December (Fig. 3.1b). In January and February, SWC was stable at 0.067  $\pm$  0.005 m<sup>3</sup> m<sup>-3</sup>, and then gradually increased in March with the thaw of frozen soil.

Soil temperature measured simultaneously with flux measurement was not significantly different between positions nor between treatments (Table 3.1). Meanwhile, SWC was significantly lower at Far positions (p < 0.05).



Fig. 3.1. Temporal variations in daily means of soil temperature ( $T_s$ ) at a depth of 6 cm (a) and volumetric soil water content (SWC) at a depth of 3 cm (b) measured at a nearby station 150 m from the study site from May 2017 to August 2018.

**Table 3.1.** Soil temperature ( $T_s$ ) at a depth of 5 cm and volumetric soil water content (SWC) of the top 5-cm soil measured in each collar immediately after CO<sub>2</sub> efflux measurement. Means ( $\pm$  1 standard deviation) were shown in each cell. Two-way repeated measure ANOVA was applied.

Position	Position Treatment		SWC $(m^3 m^{-3})$
Noor	Control	$20.4\pm7.1$	$0.097\pm0.024$
Ineal	Trenched	$19.5\pm6.9$	$0.088\pm0.018$
For	Control	$20.7\pm7.4$	$0.079\pm0.022$
Far	Trenched	$19.8\pm7.2$	$0.080\pm0.016$
	Position	0.89	0.018
ANOVA (p-value)	Treatment	0.65	0.44
	Interaction	0.99	0.36

## **3.1.2.** Soil CO<sub>2</sub> efflux

Soil CO<sub>2</sub> efflux ( $R_c$ ) was measured three and 15 times, respectively, before and after trenching in early July 2017 (Fig. 3.2). Overall,  $R_c$  varied according to a seasonal variation in  $T_s$  (Fig. 3.1). Before trenching, no significant difference was found between CC and TC in all pairs (p > 0.05), which suggested that initial  $R_c$  was almost the same in each pair. Meanwhile, significant difference was found between the two collars in all pairs (p < 0.05) after trenching. Mean  $R_c$  after trenching was 2.41 ± 1.50 (CC) and 1.54 ± 1.11 (TC) µmol m<sup>-2</sup> s<sup>-1</sup> at Near and 1.69 ± 0.90 (CC) and 1.30 ± 0.70 (TC) µmol m<sup>-2</sup> s<sup>-1</sup> at Far. The  $R_c$  showed a significant positive exponential relationship with  $T_s$  (Eq. 2) on each collar (p < 0.05). Figure 3.3 resulted from mean  $R_c$  on each measuring date after trenching. The  $Q_{10}$  values were 2.01 (CC) and 2.39 (TC) at Near and 1.58 (CC) and 1.62 (TC) at Far for  $T_s$  at a depth of 5 cm. In contrast, no significant linear nor curvilinear relationship was found between temperature-normalized  $R_c$  ( $R_b$ , Eq. 3) and SWC (data not shown). Thus, we estimate  $R_{CC}$  and  $R_{TC}$  half hourly for every pair of collars from  $T_s$  monitoring data using Eq. 2.

The result of the root litter bag experiment is shown in Fig. 3.4. A negative exponential equation (Eq. 4) was fitted to the data in 2017 and 2018 separately, because no change was measured in the winter between mid-November and mid-May. The *k* values before mid-November and after mid-May were  $2.1 \times 10^{-3}$  ( $r^2 = 0.88$ ) and  $1.7 \times 10^{-3}$  d<sup>-1</sup> ( $r^2 = 0.97$ ), respectively. We set *k* at 0 in the winter. If the equation is fitted to all data, *k* is calculated to be  $1.00 \times 10^{-3}$  d<sup>-1</sup> ( $r^2 = 0.71$ ), which is equivalent to 0.36 yr<sup>-1</sup>.

Daily  $R_{DR}$  was calculated from the k values using Eq. 5, and then daily  $R_s$  ( $R_{CC}$ ) was partitioned into  $R_h$  and  $R_r$  of all roots (Fig. 3.5). Since  $R_{CC}$  and  $R_{TC}$  were estimated from  $T_s$ , all respiration components varied according to the seasonal variation of  $T_s$  (Fig. 3.1). Under snow,  $R_s$ ,  $R_h$  and  $R_r$  were stable at 0.61, 0.37 and 0.24 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, at Near and 0.42, 0.33 and 0.08 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, at Far, which indicates that the ratios of  $R_r$  to  $R_s$  were 0.39 and 0.19, respectively, at Near and Far under snow. The  $R_m$  of coarse roots ( $R_m_{coarse}$ ) was calculated using Eq. 6. Thus, the difference between  $R_r$  and  $R_m_{coarse}$  correspond to  $R_r$  of fine roots, which were 0.20 and 0.04 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, at Near and Far under snow. Annual CO<sub>2</sub> efflux was calculated between August 2017 and July 2018 (Table 3.2). All the CO<sub>2</sub> effluxes were larger at Near than at Far, though only  $R_{CC}$  was significantly different (p < 0.05). The ratios (Far / Near) of  $R_{CC}$  ( $R_s$ ),  $R_h$  and  $R_r$  were 0.63, 0.83 and 0.28, respectively. The  $R_{DR}$  accounted for 3% of  $R_s$  at both positions. The  $R_r$  accounted for 37 and 16% of  $R_s$ , respectively, at Near and Far.

Similarly, measured  $R_{CC}$  was partitioned into  $R_h$  and  $R_r$  for each collar pair. The  $R_h$  and  $R_r$  significantly responded to  $T_s$  (p < 0.05) according to Eq. 2. The  $Q_{10}$  values of  $R_h$  and  $R_r$  were 2.02 ± 1.28 and 2.01 ± 1.21 (mean ± 1 SD), respectively, at Near (n = 6) and 2.15 ± 1.20 and 2.14 ± 1.29, respectively, at Far (n = 4). No significant difference was found between  $R_h$  and  $R_r$  at both positions.



**Fig. 3.2.** Temporal variations in soil CO<sub>2</sub> efflux ( $R_c$ ) in control collars (CC) and trenched collars (TC) at Near (n = 6) (a) and Far (n = 4) (b) from May 2017 to August 2018. Vertical bars denote 1 standard deviation. The dashed vertical lines denote the time of trenching.



**Fig. 3.3.** Relationship of soil CO<sub>2</sub> efflux ( $R_s$ ) with soil temperature ( $T_s$ ) in collars for root sampling (CC) and trenched collars (TC) at Near (n = 6) (a) and Far (n = 4) (b) from May 2017 to August 2018. An exponential curve (Eq. 1) was significantly fitted;  $r^2$  values were shown.



**Fig. 3.4.** Temporal variation in fine root litter left in root litter bags (remainder ratio) from July 2017 to October 2018. Means ( $\pm 1$  standard deviation) were plotted (n = 10). A negative exponential equation (Eq. 4) was separately fitted to data in 2017 and 2018. No change was found in fine root litter between mid-November 2017 and mid-May 2018 (dashed line).



Fig. 3.5. Temporal variations in mean daily soil respiration ( $R_s$ ), heterotrophic respiration ( $R_h$ ), root respiration ( $R_r$ ) and coarse root maintenance respiration ( $R_{m\_coarse}$ ) at Near (n = 6) (a) and Far (n = 4) (b) calculated from the monitoring data of soil temperature ( $T_s$ ) between August 2017 and July 2018.

**Table 3.2.** Annual soil respiration ( $R_{CC}$  or  $R_s$ ), soil CO<sub>2</sub> efflux in trenched collars ( $R_{TC}$ ), CO<sub>2</sub> emissions through dead root decomposition in trenched collars ( $R_{DR}$ ), heterotrophic respiration ( $R_h$ ) and root respiration ( $R_r$ ) (g C m<sup>-2</sup> yr<sup>-1</sup>) at Near (n = 6) and Far (n = 4). Means (± 1 standard deviation) were shown. Numbers in parentheses denote percentages against  $R_{CC}$ .

Position	$R_{\rm CC}(R_{\rm s})$	$R_{ m TC}$	$R_{\rm DR}$	$R_{ m h}$	$R_{ m r}$
Near	493 ± 111 (100)	$326 \pm 75 (66)$	13 (3)	$313 \pm 75 (63)$	181 ± 123 (37)
Far	311 ± 68 (100)	$269 \pm 79$ (86)	8 (3)	261 ± 79 (84)	51 ± 34 (16)

#### 3.1.3. Biomass and production of fine roots

Fine root biomass  $(B_{\rm f})$  at Near showed a small peak in early September 2017, and then tended to increase from November until late August 2018 (Fig. 3.6a). Similarly,  $B_{\rm f}$  at Far showed an increasing tendency in the warm season. In contrast, fine root production  $(P_{\rm f})$ showed a clear seasonal variation both at Near and Far (Fig. 3.6b); it decreased during the fall and increased during the late spring and summer. In the cold season between mid-November and mid-May, including the snowy season for about four months,  $P_{\rm f}$  was small at  $0.035 \pm 0.018$ and 0.043  $\pm$  0.041 g m<sup>-2</sup> d<sup>-1</sup>, respectively, at Near and Far. The  $P_{\rm f}$  showed a significant exponential relationship (p < 0.05) with  $T_s$  at a depth of 5 cm in the same form as Eq. 2 (Fig. 3.7a), which resulted in  $Q_{10}$  values of 4.48 and 2.51, respectively, at Near and Far. The high  $Q_{10}$  of 4.48 reflects a large seasonal variation in  $P_{\rm f}$  at Near (Fig. 3.6b). Also,  $B_{\rm f}$  showed a significant exponential relationship (p < 0.05), though  $Q_{10}$  values were lower with 1.08 and 1.49, respectively, at Near and Far. A significant linear relationship with a slope of 1.20 ( $r^2 =$ 0.43, p < 0.001) was found between  $R_r$  and  $P_f$ , regardless of positions (Fig. 3.8a). The relationship between  $R_r$  and  $B_f$  was also significant ( $r^2 = 0.33$ , p < 0.01) (Fig. 3.8b), whereas  $P_f$ was not significantly correlated with  $B_{\rm f}$  (p > 0.10). Annually,  $P_{\rm f}$  (p < 0.05) and mean  $B_{\rm f}$  (p <0.01) were significantly larger at Near than Far, whereas the turnover rate (p < 0.01) was significantly lower at Near (Table 3.3).



**Fig. 3.6.** Temporal variations in fine root biomass ( $B_f$ ) (a) and fine root production ( $P_f$ ) (b) at Near (n = 6) and Far (n = 4) from July 2017 to August 2018. Means ( $\pm 1$  standard deviation) were shown.



**Fig. 3.7.** Relationships of fine root production ( $P_f$ ) with soil temperature ( $T_s$ ) (a) and fine root biomass ( $B_f$ ) with soil temperature ( $T_s$ ) (b) at Near (n = 6) and Far (n = 4). Soil temperature was averaged for each interval of root sampling. Means ( $\pm 1$  standard deviation) were plotted. An exponential curve (Eq. 1) was significantly fitted.



**Fig. 3.8.** Relationship of root respiration ( $R_r$ ) with fine root production ( $P_f$ ) (a) or fine root biomass ( $B_f$ ) (b) at Near and Far. Root respiration was averaged for each interval of root sampling. A significant regression line was drawn for each scatter plot.

**Table 3.3.** Annual values of fine root production ( $P_f$ ), annual increase in fine root biomass ( $\Delta B_f$ ), fine root mortality ( $M_f$ ), mean fine root biomass ( $B_f$ ) and fine root turnover rates at Near (n = 6) and Far (n = 4). Means ( $\pm 1$  standard deviation) were shown.

Posotion	$P_{\rm f}$ (g m <sup>-2</sup> y <sup>-1</sup> )	$\Delta B_{\rm f} \ ({ m g m}^{-2} { m y}^{-1})$	$M_{\rm f}$ (g m <sup>-2</sup> y <sup>-1</sup> )	$\frac{\text{Mean } B_{f}}{(g \text{ m}^{-2})}$	Turnover rate (y <sup>-1</sup> )
Near	$81\pm22$	$12 \pm 33$	$93\pm47$	$70\pm21$	$1.27\pm0.57$
Far	$41 \pm 10$	$4\pm5$	$46\pm10$	$13 \pm 5$	$3.26 \pm 1.45$

#### **3.1.4.** Partitioning root respiration

Model 1 (Eq. 6) was significantly fitted to the data set (p < 0.001) with an adjusted  $r^2$  of 0.59, respectively (Table 3.4). Using the parameters, we partitioned  $R_r$ .

The  $Q_{10}$  calculated from the parameter f was 1.7. Annual  $R_r$  was partitioned into  $R_g$  and  $R_m$  using the fitting parameters (Table 3.5). At Near,  $R_g$  of fine roots,  $R_m$  of fine roots and  $R_m$  of coarse roots accounted for 26, 57 and 17% of their sum (estimated  $R_r$ ), respectively. The bias between  $R_r$  from Table 3.2 and the estimated  $R_r$  was zero. Meanwhile,  $R_g$ , fine root  $R_m$  and coarse root  $R_m$  accounted for 34, 27 and 39% of their sum at Far; the bias was 19 g C m<sup>-2</sup> yr<sup>-1</sup> (37%). The larger bias at Far was partly due to a smaller number of replications at Far (n = 4) than at Near (n = 6). The  $R_g$  and fine root  $R_m$  were significantly larger at Near (p < 0.05). In addition, the respirations were spatially averaged with a weight of circumference: 3.14 and 6.28 m for Near and Far, respectively. As a result,  $R_g$ , fine root  $R_m$  and coarse root  $R_m$  accounted for 30, 44 and 26% of the total  $R_r$  on average in a circumferential area between 0.5 and 1.0 m in radius.

The  $R_g$  varied seasonally in parallel with  $P_f$  (Figs. 6b and 9), whereas  $R_m$  varied mainly according to a seasonal variation in  $T_s$  (Figs. 3.1 and 3.9). The contribution of  $R_g$  to fine root  $R_r$  (=  $R_g / (R_g + \text{fine root } R_m)$ ) was larger at Far with 0.39 in the cold season and 0.59 on average in the warm season than at Near with 0.10 and 0.35, respectively (Fig. 3.9c).

	Parameter	<i>p</i> -value
<i>c</i> (g C g DM <sup>-1</sup> )	$0.57\pm0.22$	0.012
$d (g C g DM^{-1} d^{-1})$	$0.0021 \pm 0.00087$	0.022
$f(^{\circ}\mathrm{C}^{-1})$	$0.054\pm0.021$	0.016
Adjusted $r^2$	0.59	
<i>p</i> -value	< 0.001	

**Table 3.4.** Fitting parameters of Eq. 6 (n = 50) ( $\pm 1$  standard error) with significance (p value).

**Table 3.5.** Annual sums (g C m<sup>-2</sup> yr<sup>-1</sup>) of root respiration ( $R_r$ ) from Table 3.2, fine root growth respiration ( $R_g$ ), fine root maintenance respiration ( $R_{m_fine}$ ), coarse root maintenance respiration ( $R_{m_coarse}$ ) and the sum of  $R_g$ ,  $R_{m_fine}$  and  $R_{m_coarse}$  (Sum) at Near (n = 6) and Far (n = 4), which corresponds to  $R_r$ . Values at Near and Far were spatially averaged with a weight of circumference: 3.14 and 6.28 m for Near and Far, respectively (weighted average). Means ( $\pm 1$  standard deviation) were shown. Numbers in parentheses denote percentages against Sum.

Position	$R_{ m r}$	$R_{ m g}$	$R_{\rm m\_fine}$	$R_{\rm m\_coarse}$	Sum
Near	$181\pm123$	$47\pm12$	$104\pm31$	30 (17)	$181\pm32$
		(26)	(57)		(100)
Far	$51 \pm 34$	24 ± 3 (34)	$19 \pm 4$ (27)	27 (39)	$70 \pm 4 \ (100)$
Weighted average	94	32 (30)	47 (44)	28 (26)	107 (100)



**Fig. 3.9.** Temporal variations in fine root growth respiration ( $R_g$ ) and maintenance respiration ( $R_{m_{fine}}$ ) at Near (a, n = 6) and Far (b, n = 4), and the ratio of  $R_g$  to the sum of  $R_g$  and  $R_m$  (c) from July 2017 to August 2018. Means ( $\pm 1$  standard deviation) were shown.

#### 3.2 Experiment 2 (2019–2020)

## **3.2.1 Environmental conditions**

During the annual period between November 2019 and October 2020, mean air temperature and total precipitation were 8.7°C and 968 mm at the Tomakomai meteorological station. The annual mean air temperature was high beyond the range of its decadal mean  $\pm 1$  SD (8.1  $\pm$ 0.3°C) between 2011 and 2020, whereas annual precipitation was low beyond the decadal range (1305  $\pm$  202 mm yr<sup>-1</sup>). The  $T_s$  at a depth of 6 cm showed an obvious seasonal variation with the maximum of 24.8°C in mid-August 2020 and the minimum of -5.0°C in early January 2020 on a daily basis (Fig. 3.10a). The lower minimum  $T_s$  was caused by thin snow accumulation until January. The SWC at 3 cm rapidly decreased by soil freezing in December (Fig. 3.10b). In January and February, SWC was stable at 0.045  $\pm$  0.003 m<sup>3</sup> m<sup>-3</sup>, and then gradually increased in March. At the end of March, SWC rapidly increased with the thaw of frozen soil.

Soil temperature measured simultaneously with flux measurement was not significantly different between positions nor between treatments (Table 3.1). Meanwhile, SWC was significantly lower in trenched collars (TC) (p < 0.05).



**Fig. 3.10.** Temporal variations in daily means of soil temperature  $(T_s)$  at a depth of 6 cm (a) and volumetric soil water content (SWC) at a depth of 3 cm (b) measured at a nearby station 150 m from the study site from September 2019 and November 2020.

**Table 3.6.** Soil temperature ( $T_s$ ) at a depth of 5 cm and volumetric soil water content (SWC) of the top 5-cm soil measured in each collar immediately after CO<sub>2</sub> efflux measurement (n = 9). Means (± 1 standard deviation) were shown in each cell. Two-way repeated measure ANOVA was applied.

Position	Treatment	$T_{\rm s}$ (°C)	SWC $(m^3 m^{-3})$	
Neer	Control	$15.4\pm6.3$	$0.119\pm0.051$	
Inear	Trenched	$15.1 \pm 6.2$	$0.110\pm0.045$	
Ean	Control	$15.3 \pm 6.7$	$0.119\pm0.042$	
rar	Trenched	$15.1 \pm 6.4$	$0.112\pm0.038$	
	Position	0.96	0.73	
ANOVA (p-value)	Treatment	0.59	0.035	
	Interaction	0.97	0.71	

## 3.2.2. Soil CO<sub>2</sub> efflux

Soil CO<sub>2</sub> efflux ( $R_c$ ) was measured 16 times. Overall,  $R_c$  varied according to a seasonal variation in  $T_s$  (Fig. 3.11). Significant difference was found between the two collars in all pairs (p < 0.05). In all collars,  $R_c$  was significantly related to  $T_s$  according to Eq. 2 (p < 0.05). The base  $R_c$  at 0°C ( $R_0$ ) was significantly different both between positions and between treatments (p < 0.05) (Table 3.7). The  $R_0$  was larger at Near and in CC. Meanwhile,  $Q_{10}$  was not significantly different between positions nor between treatments. In contrast, no significant linear nor curvilinear relationship was found between temperature-normalized  $R_c$  ( $R_b$ , Eq. 3) and SWC (Fig.3.12). Thus, we estimate  $R_{CC}$  and  $R_{TC}$  half hourly for every pair of collars from  $T_s$  monitoring data using Eq. 2.

Daily  $R_{DR}$  was calculated from the *k* values of  $1.00 \times 10^{-3} \text{ d}^{-1}$  for fine roots (Fig. 3.13) using Eq. 5, and then daily  $R_s$  ( $R_{CC}$ ) was partitioned into  $R_h$  and  $R_r$  of all roots (Fig. 3.14). Although *k* values are different between fine and coarse roots (Sun *et al.*, 2020), we used the *k* both for fine and coarse roots.

Since  $R_{CC}$  and  $R_{TC}$  were estimated from  $T_s$ , all respiration components varied according to the seasonal variation of  $T_s$  (Fig. 3.10). Under snow,  $R_s$ ,  $R_h$  and  $R_r$  were stable at 0.72, 0.39 and

0.33 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, at Near and 0.61, 0.28 and 0.33 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, at Far, which indicates that the ratios of  $R_r$  to  $R_s$  were 0.46 and 0.55, respectively, at Near and Far under snow. The  $R_m$  of coarse roots ( $R_{m\_coarse}$ ) was calculated using Eq. 6. Thus, the difference between  $R_r$  and  $R_{m\_coarse}$  correspond to  $R_r$  of fine roots, which were 0.68 and 0.59 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, at Near and Far under snow. Annual CO<sub>2</sub> efflux was calculated between November 2019 and October 2020 (Table 3.8). The ratios (Far / Near) of  $R_{CC}$  ( $R_s$ ),  $R_h$  and  $R_r$  were 0.78, 0.81 and 0.74, respectively. The total  $R_{DR}$  accounted for 45–47% of  $R_s$ .



**Fig. 3.11.** Temporal variations in soil CO<sub>2</sub> efflux ( $R_c$ ) in control collars (CC) and trenched collars (TC) at Near and Far (n = 9) from August 2019 to November 2020. Vertical bars denote 1 standard deviation.

Position	Position Treatment		$Q_{10}$
Neer	Control	$0.73\pm0.18$	$1.97\pm0.43$
Ineal	Trenched	$0.49 \pm 0.15$	$1.78\pm0.28$
For	Control	$0.49 \pm 0.17$	$2.13\pm0.41$
гаг	Trenched	$0.34\pm0.16$	$2.04\pm0.52$
	Position	0.001	0.139
ANOVA (p-value)	Treatment	0.001	0.317
	Interaction	0.404	0.763

**Table 3.7.** Base soil respiration at 0°C ( $R_0$ ) and  $Q_{10}$  determined by Eq. 2. Means ± 1 standard deviation (n = 9) were shown. Two-way repeated measure ANOVA was applied.



Fig. 3.12. Temperature-normalized  $R_c$  (at 15°C) vs. soil moisture (SWC at 3 cm depth).



**Fig. 3.13.** Temporal variation in fine root litter left in root litter bags (remainder ratio) from July 2017 to October 2018. Means  $\pm 1$  standard deviation were plotted (n = 10). A negative exponential equation (Eq. 4) was fitted to data in 2017 and 2018. The data are the same as in Fig. 3.4.



**Fig. 3.14.** Temporal variations in mean daily soil respiration ( $R_s$ ), heterotrophic respiration ( $R_h$ ), root respiration ( $R_r$ ) at Near and Far (n = 9) calculated from the monitoring data of soil temperature ( $T_s$ ) between September 2019 and November 2020.

**Table 3.8.** Annual soil respiration ( $R_{CC}$  or  $R_s$ ), soil CO<sub>2</sub> efflux in trenched collars ( $R_{TC}$ ), CO<sub>2</sub> emissions through dead root decomposition in trenched collars ( $R_{DR_{fine}}$ ,  $R_{DR_{coarse}}$  and  $R_{DR_{all}}$ ), heterotrophic respiration ( $R_h$ ) and root respiration ( $R_r$ ) (g C m<sup>-2</sup> yr<sup>-1</sup>) at Near and Far (n = 9) between November 2019 and October 2020. Means  $\pm 1$  standard deviation were shown. Numbers in parentheses denote percentages against  $R_{CC}$ .

Position	$R_{\rm CC}(R_{\rm s})$	$R_{ m TC}$	$R_{\rm DR}$ fine	$R_{\rm DR\_coarse}$	$R_{ m DR\_all}$	$R_{ m h}$	$R_{ m r}$
Near	$610\pm78$	$356\pm73$	$27 \pm 14$	$6\pm 6$	$33\pm14$	$323\pm74$	$287\pm86$
	(100)	(58)	(4.4)	(1.0)	(5.4)	(53)	(47)
Far	$474 \pm$	$282 \pm 84$	$17 \pm 18$	$3\pm4$	$20\pm20$	$262 \pm 71$	$212 \pm 104$
	155 (100)	(59)	(3.5)	(0.6)	(4.1)	(55)	(45)

## 3.2.3. Biomass and production of fine roots

Fine root biomass ( $B_f$ ) at Near showed a small peak in October 2019 and May–July 2020 (Fig. 3.15). Similarly,  $B_f$  at Far showed a small peak in September–October 2019 and July 2020, though seasonal variation was not so clear. In contrast, fine root production ( $P_f$ ) showed a clear seasonal variation both at Near and Far (Fig. 3.16); it sharply decreased in November 2019 and increased in April 2020. In 2020,  $P_f$  peaked in early summer and decreased during fall. In the cold season between mid-November and mid-April,  $P_f$  was small at 0.11 ± 0.02 and 0.13 ± 0.21 g m<sup>-2</sup> d<sup>-1</sup>, respectively, at Near and Far.

Neither  $B_f$  nor  $P_f$  showed no significant correlation (p > 0.05) with  $T_s$  (Fig. 3.17). The  $R_r$  showed a significant linear relationship both with  $B_f$  ( $r^2 = 0.16$ , p < 0.001) and  $P_f$  ( $r^2 = 0.24$ , p < 0.001), regardless of positions (Fig. 3.18). The relationship between  $P_f$  and  $B_f$  was also significant ( $r^2 = 0.12$ , p < 0.01) (data not shown). Annually, only the turnover rate calculated as the ratio of  $P_r$  and Mena  $B_f$  showed significant difference between positions, which was significantly larger at Far (p < 0.05) (Table 3.9).



**Fig. 3.15.** Temporal variations in fine root biomass ( $B_f$ ) at Near and Far (n = 9) from September 2019 to November 2020. Means  $\pm 1$  standard deviation were shown.



**Fig. 3.16.** Temporal variations in fine root production ( $P_f$ ) at Near and Far (n = 9) from September 2019 to November 2020. Means  $\pm 1$  standard deviation were shown.



Fig. 3.17. Relationships of fine root biomass  $(B_f)$  with soil temperature  $(T_s)$  (a) and fine root production  $(P_f)$  with soil temperature  $(T_s)$  (b) at Near and Far (n = 9). Soil temperature was averaged for each interval of root sampling. Means  $\pm 1$  standard deviation were plotted.



**Fig. 3.18.** Relationship of root respiration  $(R_r)$  with fine root biomass  $(B_f)$  (a) or fine root production  $(P_f)$  (b) at Near and Far. Root respiration was averaged for each interval of root sampling. A significant regression line was drawn.

**Table 3.9.** Annual values of fine root production ( $P_f$ ), annual increase in fine root biomass ( $\Delta B_f$ ), fine root mortality ( $M_f$ ), mean fine root biomass ( $B_f$ ), mean coarse root biomass ( $B_c$ ) and fine root turnover rates at Near and Far (n = 9) between November 2019 and October 2020. Means  $\pm 1$  standard deviation were shown.

Posotion	$P_{\rm f}$ (g m <sup>-2</sup> y <sup>-1</sup> )	$\frac{\Delta B_{\rm f}}{({\rm g m}^{-2} {\rm y}^{-1})}$	$M_{\rm f}$ (g m <sup>-2</sup> y <sup>-1</sup> )	Mean $B_{\rm f}$ (g m <sup>-2</sup> )	Turnover rate (y <sup>-1</sup> )	Mean $B_c$ (g DMm <sup>-2</sup> )
Near	$115\pm20$	$3\pm48$	$112\pm98$	$133\pm40$	$0.90\pm0.31$	$44\pm42$
Far	$102\pm29$	$4\pm 26$	$98\pm 33$	$78\pm43$	$1.67\pm0.89$	$20\pm26$

#### 3.2.4. Sap flow

Sap flow rates ( $T_r$ ) normalized by fine root biomass were measured form July of 2019 to November of 2020 with a suspension of five months in the leafless season. Daily  $T_r$  showed a similar seasonal variation with that of Ts (Fig. 3.19). During the leafless season between November and April,  $T_r$  would have been zero.



Fig. 3.19. Daily sap flow rates per fine root biomass ( $T_r$ ). Mean of five-day moving average is shown (n = 3)

#### **3.2.5.** Partitioning root respiration

Model 1 (Eq. 6) and Model 2 (Eq. 7) were significantly fitted to the data set (p < 0.001) with an adjusted  $r^2$  of 0.51 and 0.53, respectively (Table 3.11). All parameters were also significantly determined (p < 0.05). The parameters related to  $R_m$  (d and f) were almost the same for the models. The  $Q_{10}$  derived from f was 2.46–2.61. However, the parameter related to  $R_g$  (c) was smaller in Model 2 by 18%; the difference was due to adding  $R_{ion}$ . Part of  $R_g$  was distributed into  $R_{ion}$ .

We partitioned  $R_r$  using Model 2, because  $r^2$  was slightly larger in Model 2 (Table 3.12). Annually, qt Near,  $R_g$  of fine roots,  $R_m$  of fine roots,  $R_{ion}$  and  $R_m$  of coarse roots accounted for 27, 48, 10 and 15% of their sum (estimated  $R_r$ ), respectively. The bias between  $R_r$  from Table 3.8 and the estimated  $R_r$  was 5 g C m<sup>-2</sup> yr<sup>-1</sup> (2%). Meanwhile,  $R_g$  of fine roots,  $R_m$  of fine roots,  $R_m$  of fine roots,  $R_m$  of coarse roots accounted for 37, 43, 9 and 12% of their sum at Far; the bias was 35 g C m<sup>-2</sup> yr<sup>-1</sup> (20%). The respirations were spatially averaged with a weight of circumference: 3.14 and 6.28 m for Near and Far, respectively. As a result,  $R_g$  of fine roots,  $R_m$  of fine roots,  $R_m$  of fine roots,  $R_m$  of and  $R_m$  of coarse roots accounted for 32, 46, 9 and 13% of the total  $R_r$  on average in a circumferential area between 0.5 and 1.0 m in radius.

The  $R_g$  varied seasonally in parallel with  $P_f$  (Fig. 3.16), whereas  $R_m$  varied mainly according to a seasonal variation in  $T_s$  (Fig. 3.10a). Thus,  $R_g$  peaked earlier than  $R_m$ .

	Model 1 (Eq. 6)	)	Model 2 (Eq. 7)		
	Parameter	<i>p</i> -value	Parameter	<i>p</i> -value	
<i>c</i> (g C g DM <sup>-1</sup> )	$0.73\pm0.13$	< 0.001	$0.60\pm0.14$	< 0.001	
d (g C g DM <sup>-1</sup> d <sup>-1</sup> )	$0.00081 \pm 0.00025$	0.001	$0.00080 \pm 0.00026$	0.002	
$f(^{\circ}\mathrm{C}^{-1})$	$0.096\pm0.014$	< 0.001	$0.090\pm0.015$	< 0.001	
g (g C g H <sub>2</sub> O <sup>-1</sup> )			$0.00039 \pm 0.00018$	0.033	
Adjusted $r^2$ 0.51			0.53		
<i>p</i> -value	< 0.001		< 0.001		

**Table 3.11.** Fitting parameters of Eqs. 6 and 7 (n = 144) ( $\pm 1$  standard error) with significance (p value).



**Fig. 3. 20.** Seasonal variations of fine root growth respiration ( $R_g$ ), fine root maintenance respiration ( $R_{m_fine}$ ), ion uptake respiration ( $R_{ion}$ ) and coarse root maintenance respiration ( $R_{m_coarse}$ ) at Near and Far (n = 9) calculated using Model 2 between September 2019 and November 2020. Means  $\pm 1$  standard deviation are shown

**Table 3.12.** Annual sums (g C m<sup>-2</sup> yr<sup>-1</sup>) of root respiration ( $R_r$ ) from Table 3.8, fine root growth respiration ( $R_g$ ), fine root maintenance respiration ( $R_{m_fine}$ ), ion uptake respiration ( $R_{ion}$ ), coarse root maintenance respiration ( $R_{m_coarse}$ ) and the sum of  $R_g$ ,  $R_{m_fine}$ ,  $R_{ion}$  and  $R_{m_coarse}$  (Sum) at Near and Far (n = 9), which corresponds to  $R_r$ . Values at Near and Far were spatially averaged with a weight of circumference: 6.28 and 9.42 m for Near and Far, respectively (weighted average). Means ±1 standard deviation are shown. Numbers in parentheses denote percentages against Sum.

Position	$R_{ m r}$	$R_{ m g}$	$R_{\rm m\_fine}$	$R_{\rm ion}$	$R_{\rm m\_coarse}$	Sum
Near	$\begin{array}{c} 287\pm86\\ (98)\end{array}$	$78 \pm 16$ (27)	$141 \pm 41$ (48)	$28 \pm 8$ (10)	$45 \pm 43$ (15)	$292 \pm 73$ (100)
Far	$\begin{array}{c} 212\pm104\\(120)\end{array}$	$65 \pm 18$ (37)	$76 \pm 49$ (43)	$15 \pm 10$ (9)	$21 \pm 26$ (12)	$\begin{array}{c} 177\pm97\\(100)\end{array}$
Weighted average	237 (110)	69 (32)	98 (46)	19 (9)	29 (13)	215 (100)

## 4. Discussion

In the previous study conducted in the same area (Sun *et al.*, 2020), fine root dynamics and soil CO<sub>2</sub> efflux were measured simultaneously within the same collar over a year in adjacent larch-dominated deciduous (DF) and evergreen conifer (EF) forests, annual  $R_g$  and  $R_m$  of tree fine roots were quantified on an annual basis using a multiple regression model. However, the results would include considerable uncertainty because of relatively complex experimental conditions in the field, which arose from forest maturity and rich understory species. The mature forests were relatively rich in SOM, litter accumulation and roots. Thus, the forests had a large spatial variation in  $R_h$  originating from SOM and litter accumulation. The large spatial variation made it difficult to separate  $R_s$  into  $R_h$  and  $R_r$  in each pair of collars, because  $R_h$  was not presumed to be identical even in two neighboring collars. Consequently, representative  $R_r$  was determined from the averages of  $R_s$  and  $R_h$  in each forest, resulting in a small number of available data for curve fitting (n = 5). In addition, the DF had canopy gaps caused by a windstorm, and consequently was rich in herbaceous plants. Thus, in the mature forests, a good portion of  $R_r$  originated from coarse roots and herbaceous roots, not fine roots targeted for the study.

In this study, we conducted a similar field experiment over a year twice in a larchdominated young forest developing on the bare ground after removing surface organic soil along with coarse woody debris to obtain robust results by simplifying experimental conditions and improving the model. The study site was poor in SOM and litter accumulation. Soil C concentration was about 30% of that in DF and showed a decreasing tendency with a distance from tree stems, which was probably caused by the gradient of litter fall. Thus, we believe that  $R_h$  was almost equal in each pair by setting two collars on a concentric fashion from an isolated larch tree, which was supported by the fact that  $R_s$  was not significantly different in each pair before trenching (Fig. 3.2). Coefficients of variance (CV) in annual  $R_h$  were 24% (Near) and 30% (Far) in Experiment 1 (Table 3.8) and 23% (Near) and 27% (Far) in Experiment 2 (Table 3.2), which were much smaller than those of DF (59%) and EF (39%). The small CV also supported the smaller spatial variation in  $R_h$ . As a result, we were able to calculate  $R_r$  in each pair and prepare 50 and 144 data sets in total for curve fitting, respectively, in Experiment 1 and 2. By separating positions into Near (0.5 m) and Far (1.0 m), we were able to use a wide range of data and make significant fitting (Tables 3.4 and 3.11). In addition, understory vegetation was very scarce, and the density of coarse roots ( $\geq 2 \text{ mm}$ ) was low with 44 ± 42 and  $20 \pm 26$  g m<sup>-2</sup>, respectively, at Near and Far in Experiment 2 (Table 3.9). The contribution of fine roots to total  $R_r$  was large at 83–85% at Near (Tables 3.5 and 3.12), whereas it was 45% and 68%, respectively, at DF and EF. In Sun *et al.* (2020), the large  $R_r$  of coarse roots and herbaceous roots (55 and 32%) were treated as a residual without seasonality. In this study, we related coarse root biomass  $(B_c)$  with  $T_s$  to estimate  $R_m$ . Since root density was low (Tables 3.3) and 3.9), annual CO<sub>2</sub> emissions through dead root decomposition ( $R_{DR}$ ) was also small at 13 (Near) and 8 (Far) g C m<sup>-2</sup> yr<sup>-1</sup> in Experiment 1 (Table 3.2) and 33 (Near) and 20 (Far) g C m<sup>-2</sup> yr<sup>-1</sup> in Experiment 2 (Table 3.8), which accounted only 3-5% of  $R_s$ . The small  $R_{DR}$  contributed to decrease uncertainty in estimating  $R_h$  (=  $R_{TC} - R_{DR}$ ) and consequently  $R_r$ . Moreover, although trenching decreases SWC owing to lack of water uptake by roots (Subke et al., 2006; Sun et al. 2020), no significant SWC decrease was found in trenched collars in Experiment 1 (Table 3.1). In contrast, SWC was significantly higher in trenched collars in Experiment 2 (Table 3.6). Anyway, the difference was small and the effect of SWC change would be negligible (Fig. 3.12).

We improved the reliability of partitioning fine root respiration, as described above. However, there is still uncertainty. Although the temperature response of coarse root  $R_m$  was incorporated into the model on the assumption that the temperature coefficient (*f*) was the same as that of fine root  $R_m$ , seasonal variation in  $B_c$  was not considered using a fixed  $B_c$  measured after Experiment 2. The  $R_g$  of coarse roots was also ignored, whereas an annual tree survey using allometric equations suggested an annual coarse root growth of 76 g m<sup>-2</sup> yr<sup>-1</sup> bewteen 2017 and 2018. The  $B_c$  was calculated to be 276 g m<sup>-2</sup> in 2017 and 352 g m<sup>-2</sup> in 2018 for larch trees, accounting for 79% of all coarse root biomass. In this study,  $B_c$  was 55 and 49 g m<sup>-2</sup>, respectively, at Near and Far in Experiment 1, which were only 14–20% of the tree survey result. The reasons for the small  $B_c$  are that biomass within 0.5 m from trees was not included, and that soil samples were taken round isolated larch trees. Wieser and Bahn (2004) continuously measured the coarse root  $R_r$  of *Pinus cembra* and reported that  $R_m$  accounted for 73–80% of the sum of  $R_m$  and  $R_g$ . Using the percentage, the  $R_g$  of coarse roots was roughly estimated from coarse root  $R_m$  to be 6–8 and 5–7 g m<sup>-2</sup> yr<sup>-1</sup>, respectively, at Near and Far, which accounted for about 15% (Near) and 25% (Far) of fine root  $R_g$  in Experiment 1 (Table 3.5). In addition, we did not directly measure ion uptake, mainly consisting of nitrogen uptake. Instead, we used water uptake by roots estimated from daily sap flow rates. It is reported that  $R_{ion}$  is proportional to water uptake in the condition of low root density, such as in our study site (Oyewole et al., 2014; Henriksson et al., 2021). Also,  $R_{ion}$  is expected to correlate with photosynthesis (Lambers *et al.*, 2008), and fine roots are mainly produced using recent photosynthate (Lynch *et al.*, 2013). Moreover, it is reported that gross primally production (GPP) is proportional to transpiration rates on a monthly basis (Scott and Biederman, 2017). Thus, sap flow rates would be proportional to ion uptake.

Since the study site was a young forest with a small amount of soil carbon, litter accumulation and roots, soil CO<sub>2</sub> efflux was considerably smaller than those of adjacent forests. For example, annual values of  $P_{\rm f}$ , mortality ( $M_{\rm f}$ ) and mean  $B_{\rm f}$  at Near were 22, 26 and 30% of those in DF, respectively, in Experiment 1 (Table 3.3). As a result, annual  $R_s$  and  $R_r$  at Near was 39 and 29% of those in DF (Table 3.2). In contrast, annual  $R_h$  at Near accounted for 74% of that in DF, even though soil carbon concentration was only 30% of that in DF. The larger  $R_{\rm h}$ than expected from carbon concentration was probably due to a difference in the decomposability of SOM. Older SOM in DF could have more recalcitrant fractions (Ryan and Law, 2005). The growth respiration coefficient ( $g_R$ ) of 0.60 g C g DM<sup>-1</sup> (Model 2 in Experiment 2) is equivalent to 50 mmol CO<sub>2</sub> g DM<sup>-1</sup> and almost doubles those in DF and EF (Table 3.11). Meanwhile, the maintenance respiration coefficients  $(g_m)$ , which is equivalent to  $d \exp(f \cdot T_s)$ , were 1.3, 3.1 and 7.6 nmol CO<sub>2</sub> g DM<sup>-1</sup> s<sup>-1</sup> at 5, 15 and 25°C, respectively (Model 2 in Experiment 2). Although the  $g_m$  at 25°C was equal to that in DF,  $g_m$  was 1.2 and 1.7 times higher at 5 and 15°C, respectively, because the base coefficient (d) was higher but the temperature coefficient (f) was lower than those in DF. In comparison with previous studies conducted in the field for Pinus taeda and Liquidambar styaciflua (George et al., 2003) and in a greenhouse for eucalyptus cuttings (Thongo M'Bou et al., 2010), the g<sub>R</sub> and g<sub>m</sub> of this study

were about ten times higher and slightly lower, respectively. The variation in the coefficients is mainly due to differences in species, environmental conditions and methods (Lambers *et al.*, 2008).

The  $Q_{10}$  values calculated from a seasonal variation in soil CO<sub>2</sub> efflux was reported to be higher in  $R_r$  than in  $R_h$  (Boone *et al.*, 1998; Wang *et al.*, 2010), because it is confounded with root phenology like in Figs. 3.6 and 3.7. Wang *et al.* (2010) also reported that the mean  $Q_{10}$ values from 13 forest sites were 3.40 and 2.42, respectively, for  $R_r$  and  $R_h$ . In this study, the  $Q_{10}$ of  $R_r$  was about 2.0 and was equivalent to that of  $R_h$ . We think that the lower  $Q_{10}$  of 2.0 was quite possible, because it was a combined result of the apparent  $Q_{10}$  values of  $P_f$  (4.48 and 2.51),  $B_f$  (1.08 and 1.49) (Fig. 3.7) and  $R_m$ . However, uncertainty might be large in  $Q_{10}$  calculation, because CO<sub>2</sub> efflux data at low temperatures were insufficient;  $Q_{10}$  is sensitive to data at low temperatures.

Soil CO<sub>2</sub> efflux was continuously estimated from the monitoring data of  $T_s$  (Figs. 3.1a and 3.10a) using an exponential equation (Eq. 2). Although constant  $R_s$  of 0.61 and 0.42 g C m<sup>-2</sup> d<sup>-1</sup> in experiment 1 were shown during the snowy season in Fig. 3.5, the  $R_s$  might be a little overestimated in comparison with winter  $R_s$  (0.5 g C m<sup>-2</sup> d<sup>-1</sup>) measured under snow in an undisturbed deciduous forest about 5 km apart from the study site (Hirano, 2005). The overestimation was probable caused by uncertainty of the parameters of Eq. 2 (Fig. 3.3), because data were lacking at low temperatures (< 10°C). In the dormant season, roots respire using NSC accumulated during the previous growing season (Furze *et al.*, 2019; Collalti *et al.*, 2020). Small  $P_f$  of about 0.04 g m<sup>-2</sup> d<sup>-1</sup> was measured both at Near and Far in the cold season from mid-November to mid-May in Experiment 1 (Fig. 3.6b). Although some studies reported fine root growth in winter (Radville *et al.*, 2016), the  $P_f$  might include spring  $P_f$  after snowmelt in early April. The fine root  $R_g$ ,  $R_m$  and  $R_{ion}$  showed obvious seasonal variations (Figs. 3.9 and 3.20), reflecting the seasonality of  $P_f$ ,  $T_s$  and  $T_r$ , respectively. At Near,  $R_g$  was smaller than  $R_m$  throughout a year (Figs. 3.9c and 3.20).

## **5.** Conclusions

In comparison with the previous study conducted in the same area, we incorporated the maintenance respiration of coarse roots and ion uptake respiration in the model for partitioning of root respiration. Moreover, we simplified the experimental conditions in the field and controlled spatial variation in heterotrophic respiration and contamination from roots other than fine roots. The improvement decreased uncertainty in experiments and considerably increased data size available for model parametrization. As a result, we succeeded in significant partitioning of root respiration and coarse root growth respiration, fine root dynamics and sap flow rates. Also, we quantified the seasonal variation of the growth, maintenance and ion uptake respirations, along with fine root dynamics. To improve the partitioning further, spatial variation in coarse root density should be considered, and more frequent measurement of fine root dynamics is given priority.

## Acknowledgment

First and foremost, I would like to express my sincere and deep gratitude to my advisor and mentor, Prof. Takashi Hirano. Without his continuous support and patient guidance throughout the entire journey of my PhD, this thesis would have never been accomplished. Apart from the learning experience of mine, life at Hokkaido University was so much easier with his constant kindness and warm support. Not only being an academic mentor, he is a moral model whom I will continue to respect and emulate for the rest of my life. What I have learned from him will keep inspiring me in the future.

I would also like to acknowledge my senior Dr. Lifei Sun for his generous assistance. He carefully demonstrated and expertly pointed many mistakes and deadlocks I have made and has saved me from taking the wrong routes in my study. When I first arrived in Japan as an international student, his help meant everything to me during difficult times.

Special thanks to Dr. Naishen Liang and Dr. Munemas Teramoto for instrumentation and sampling analysis for my research. Although we only met a few times, I could always be touched by their passion for research, and it deeply motivated me.

I would also like to extend my deepest appreciation to Prof. Ryoji Sameshima and Prof. Tomomichi Kato as the examiner of my thesis. They shared their valuable knowledge and offered much advice on my work.

It is my pleasure and fortune to thank the Hokkaido Regional Office of the Forestry Agency for allowing me to use their study site. I acknowledge the support of JPSP KAKENHI and the Environment Research and Technology Development Fund of Environmental Restoration and Conservation Agency of Japan. I humbly give my gratitude to the Government of Japan through the Japan Student Services Organization for the scholarship (The Monbukagakusho Honors Scholarship for Privately-Financed International Students).

To all members of the laboratory of Environmental and Ecological Physics, thank you for all the encouragement, care, and delight. The time spent with you is a fantastic, unforgettable, and priceless experience to me.

Last but not least, I would like to convey my thanks to my family and friends, whose mental endorsement help me go through the epidemic. I will be forever grateful for your love.

## References

- Abramoff, R. Z., and A. C. Finzi. 2015. 'Are above- and below-ground phenology in sync?', *New Phytol*, 205: 1054-61.
- Amthor, J. 2000. 'The McCree-de Wit-Penning de Vries-Thornley Respiration Paradigms: 30 Years Later', *Annals of Botany*, 86: 1-20.
- Beer, C., M. Reichstein, E. Tomelleri, P. Ciais, M. Jung, N. Carvalhais, C. Rodenbeck, M. A. Arain, D. Baldocchi, G. B. Bonan, A. Bondeau, A. Cescatti, G. Lasslop, A. Lindroth, M. Lomas, S. Luyssaert, H. Margolis, K. W. Oleson, O. Roupsard, E. Veenendaal, N. Viovy, C. Williams, F. I. Woodward, and D. Papale. 2010. 'Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate', *Science*, 329: 834-8.
- Bond-Lamberty, B., and A. Thomson. 2010. 'Temperature-associated increases in the global soil respiration record', *Nature*, 464: 579-82.
- Boone, R. D., K. J. Nadelhoffer, J. D. Canary, and J. P. Kaye. 1998. 'Roots exert a strong influence on the temperature sensitivity of soil respiration', *Nature*, 396: 570-72.
- Brunner, I., M. R. Bakker, R. G. Björk, Y. Hirano, M. Lukac, X. Aranda, I. Børja, T. D. Eldhuset,
  H. S. Helmisaari, C. Jourdan, B. Konôpka, B. C. López, C. Miguel Pérez, H. Persson, and
  I. Ostonen. 2013. 'Fine-root turnover rates of European forests revisited: an analysis of data from sequential coring and ingrowth cores', *Plant and Soil*, 362: 357-72.
- Cannell, M. G. R., and J. H. M. Thornley. 2000. 'Modeling the components of plant respiration: some guiding principle', *Annals of Botany*, 85: 45-54.
- Chapin, F. S. III, P. A. Matson, and P. M Vitousek. 2011. 'Plant carbon buddgets.' in, *Principle of terrestrial ecosystem eclogoy* (Springer: New York).
- Chen, Dima, Yang Zhang, Yongbiao Lin, Hua Chen, and Shenglei Fu. 2009. 'Stand level estimation of root respiration for two subtropical plantations based on in situ measurement of specific root respiration', *Forest Ecology and Management*, 257: 2088-97.
- Collalti, A., M. G. Tjoelker, G. Hoch, A. Makela, G. Guidolotti, M. Heskel, G. Petit, M. G. Ryan, G. Battipaglia, G. Matteucci, and I. C. Prentice. 2020. 'Plant respiration: Controlled by photosynthesis or biomass?', *Glob Chang Biol*, 26: 1739-53.
- Davidson, E. A., A. D. Richardson, K. E. Savage, and D. Y. Hollinger. 2006. 'A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated

forest', Global Change Biology, 12: 230-39.

- Epron, D. 2009. 'Separating autotrophic and heterotrophic components of soil respiration: lessons learned from trenching and related root-exclusiong experiments.' in W. Kutsch, M. Bahn and Andreas Heinemeyer (eds.), *Soil Carbon Dynamics* (Cambridge University Press: New York).
- Finér, Leena, Mizue Ohashi, Kyotaro Noguchi, and Yasuhiro Hirano. 2011. 'Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics', Forest Ecology and Management, 262: 2008-23.
- Fukuzawa K, Shibata H, Takagi K, Satoh F, Koike T, Sasa K. 2013. 'Temporal variation in fineroot biomass, production and mortality in a cool temperate forest covered with dense understory vegetation in northern Japan.', *Forest Ecol Manag* 310:700–710.
- Furze, M. E., B. A. Huggett, D. M. Aubrecht, C. D. Stolz, M. S. Carbone, and A. D. Richardson. 2019. 'Whole-tree nonstructural carbohydrate storage and seasonal dynamics in five temperate species', *New Phytol*, 221: 1466-77.
- George, K., R. J. Norby, J. G. Hamilton, and E. H. DeLucia. 2003. 'Fine-root respiration in a loblolly pine and sweetgum forest growing in elevated CO2', *New Phytologist*, 160: 511-22.
- Gholz, H. L., D. A. Wedin, S. M. Smitherman, M. E. Harmon, and W. J Parton. 2000. 'Longterm dynamics of pine and harwood litter in contrasting environments: towards a global model of decomposition', Global Change Biology, 6: 751-65.
- Granier, André. 1987. 'Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements.' *Tree physiology*, 3: 309-320.
- Hanson, P. J., N. T. Edwards, C. T. Gartedn, and J. A. Andrews. 2000. 'Separating root and soil microbial contributions to soil respiration: A review of methods and observations', *Biogeochemistry*, 48: 114-46.
- Hirano, T. 2005. 'Seasonal and diurnal variations in topsoil and subsoil respiration under snowpack in a temperate deciduous forest', *Global Biogeochemical Cycles*, 19.
- Hirano, T, R Hirata, Y Fujinuma, N Saigusa, S Yamamoto, Y Harazono, M Takada, K Inukai, and G Inoue. 2003. 'CO2 and water vapor exchange of a larch forest in northern Japan', *Tellus Series B-Chemical and Physical Meteorology*, 55: 244-57.

- Hirano, T., K. Suzuki, and R. Hirata. 2017. 'Energy balance and evapotranspiration changes in a larch forest caused by severe disturbance during an early secondary succession', *Agricultural and Forest Meteorology*, 232: 457-68.
- Hirata, Ryuichi, Takashi Hirano, Nobuko Saigusa, Yasumi Fujinuma, Koh Inukai, Yasuyuki Kitamori, Yoshiyuki Takahashi, and Susumu Yamamoto. 2007. 'Seasonal and interannual variations in carbon dioxide exchange of a temperate larch forest', *Agricultural and Forest Meteorology*, 147: 110-24.
- Janssens IA, Lankreijer H, Matteucci G, Kowalski AS, Buchmann N, Epron D, Pilegaard K, Kutsch W, Longdoz B, Grunwald T, Montagnani L, Dore S, Rebmann C, Moors EJ, Grelle A, Rannik U, Morgenstern K, Oltchev S, Clement R, Gudmundsson J, Minerbi S, Berbigier P, Ibrom A, Moncrieff J, Aubinet M, Bernhofer C, Jensen NO, Vesala T, Granier A, Schulze ED, Lindroth A, Dolman AJ, Jarvis PG, Ceulemans R, Valentini R. 2001. 'Productivity over- shadows temperature in determining soil and ecosystem res- piration across European forests.', *Glob Chang Biol* 7:269–278.
- Johnson, I. R. 1990. 'Plant respiration in relation to growth, maintenance, ion uptake and nitrogen assimilation', *Plant, Cell and Environment*, 13: 319-28.
- Lambers, H., F. S. III Chapin, and T. L. Pons. 2008. 'The role of respiration in plant carbon balance.' in, *Plant Physiological Ecology* (Springer: New York).
- Lavigne, M. B., R. J. Foster, and G. Goodine. 2004. 'Seasonal and annual changes in soil respiration in relation to soil temperature, water potential and trenching', *Tree Physiology*, 24: 415-24.
- Lynch, D. J., R. Matamala, C. M. Iversen, R. J. Norby, and M. A. Gonzalez-Meler. 2013. 'Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots', *New Phytol*, 199: 420-30.
- Makita, N., Y. Kosugi, M. Dannoura, S. Takanashi, K. Niiyama, A. R. Kassim, and A. R. Nik. 2012. 'Patterns of root respiration rates and morphological traits in 13 tree species in a tropical forest', Tree Physiol, 32: 303-12.
- Makkonen K, Helmisaari HS. 1998. 'Seasonal and yearly varia- tions of fine-root biomass and necromass in a scots pine (Pinus sylvestris L.) stand.', *Forest Ecol Manag* 102:283–290
- Marsden, C., Y. Nouvellon, A. T. M'Bou, L. Saint-Andre, C. Jourdan, A. Kinana, and D. Epron.

2008. 'Two independent estimations of stand-level root respiration on clonal Eucalyptus stands in Congo: up scaling of direct measurements on roots versus the trenched-plot technique', *New Phytol*, 177: 676-87.

- McCormack, M. L., I. A. Dickie, D. M. Eissenstat, T. J. Fahey, C. W. Fernandez, D. Guo, H. S. Helmisaari, E. A. Hobbie, C. M. Iversen, R. B. Jackson, J. Leppalammi-Kujansuu, R. J. Norby, R. P. Phillips, K. S. Pregitzer, S. G. Pritchard, B. Rewald, and M. Zadworny. 2015.
  'Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes', *New Phytol*, 207: 505-18.
- McCree, K. J. 1974. 'Equation for the rae of dark respiration of white clove and grain sorghum, as functions of dry weight, photosynthetic rate, and tempearture', *Crop Science*, 14: 509-14.
- Moyano, Fernando E., Owen K. Atkin, M. Bahn, Dan Bruhn, Andrew J. Burton, Andreas Heinemeyer, Werner L. Kutsch, and Gerhard Wieser. 2009. 'Respiration from roots and the mycorrhizosphere.' in Werner L. Kutsch, M. Bahn and Andreas Heinemeyer (eds.), *Soil Carbon Dynamics* (Cambridge University Press: New York).
- Neumann, M., D. L. Godbold, Y. Hirano, and L. Finer. 2020. 'Improving models of fine root carbon stocks and fluxes in European forests', Journal of Ecology, 108: 496-514.
- Noguchi K, Sakata T, Mizoguchi T, Takahashi M. 2005. 'Estimating the production and mortality of fine roots in a Japanese cedar (Cryptomeria japonica D. Don) plantation using a minirhizotron technique.', *J Forest Res-Jpn* 10:435–441.
- Penning de Vries, F. W. T. 1974. 'Substrate utilization and respiration in relation to growth and maintenance in higher plants', *Netherlands Journal of Agricultural Science*, 22: 40-44.
- Radville, L., M. L. McCormack, E. Post, and D. M. Eissenstat. 2016. 'Root phenology in a changing climate', *J Exp Bot*, 67: 3617-28.
- Rewald, B., A. Rechenmacher, and D. L. Godbold. 2014. 'It's complicated: intraroot system variability of respiration and morphological traits in four deciduous tree species', *Plant Physiol*, 166: 736-45.
- Richter, Daniel D., Daniel Markewitz, S. E. Trumbore, and Carol G. Wells. 1999. 'Rapid accumulation and turnover of soil carbon in a re-establishing forest', *Nature*, 400: 56-58.

Ryan, M. G., and B. E. Law. 2005. 'Interpreting, measuring, and modeling soil respiration',

Biogeochemistry, 73: 3-27.

- Sano, Tomohito, Takashi Hirano, Naishen Liang, Ryuichi Hirata, and Yasumi Fujinuma. 2010. 'Carbon dioxide exchange of a larch forest after a typhoon disturbance', *Forest Ecology* and Management, 260: 2214-23.
- Scott-Denton, Laura E., Todd N. Rosenstiel, and Russell K. Monson. 2006. 'Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration', *Global Change Biology*, 12: 205-16.
- Silver, W. L., and R. K. Miya. 2001. 'Global patterns in root decomposition: comparisons of climate and litter quality effects', *Oecologia*, 129: 407-19.
- Subke, Jens-Arne, Ilaria Inglima, and M. Francesca Cotrufo. 2006. 'Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review', *Global Change Biology*, 12: 921-43.
- Sun, L. F., M. Teramoto, N. Liang, T. Yazaki, and T. Hirano. 2017. 'Comparison of litter-bag and chamber methods for measuring CO2 emissions from leaf litter decomposition in a temperate forest', *Journal of Agricultural Meteorology*, 73: 59-67.
- Sun, Lifei, Takashi Hirano, Tomotsugu Yazaki, Munemasa Teramoto, and Naishen Liang. 2020.
   'Fine root dynamics and partitioning of root respiration into growth and maintenance components in cool temperate deciduous and evergreen forests', *Plant and Soil*, 446: 471-86.
- Sun, Qingling, Baolin Li, Tao Zhang, Yecheng Yuan, Xizhang Gao, Jinsong Ge, Fei Li, and Zhijun Zhang. 2017. 'An improved Biome-BGC model for estimating net primary productivity of alpine meadow on the Qinghai-Tibet Plateau', *Ecological Modelling*, 350: 55-68.
- Sweetlove, L. J., T. C. Williams, C. Y. Cheung, and R. G. Ratcliffe. 2013. 'Modelling metabolic CO(2) evolution--a fresh perspective on respiration', *Plant Cell Environ*, 36: 1631-40.
- Thongo M'Bou, Armel, Laurent Saint-André, Agnès de Grandcourt, Yann Nouvellon, Christophe Jourdan, Fidèle Mialoundama, and Daniel Epron. 2010. 'Growth and maintenance respiration of roots of clonal Eucalyptus cuttings: scaling to stand-level', *Plant and Soil*, 332: 41-53.
- Thornley, J. H. 2011. 'Plant growth and respiration re-visited: maintenance respiration defined

- it is an emergent property of, not a separate process within, the system - and why the respiration : photosynthesis ratio is conservative', *Ann Bot*, 108: 1365-80.

Thornley, J. H. M. 1970. 'Respiration, growth and maintenance in plants', Nature, 227: 304-05.

- Vogt, K. A., D. J. Vogt, and J. Bloomfield. 1998. 'Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level', *Plant and Soil*, 200: 71-89.
- Wieder, R. Kelman, and G. E. Lang. 1982. 'A critique of the analytical methods used in examining decomposition data obtained from litter bags', *Ecology*, 63: 1636-42.
- Wieser, Gerhard, and Michael Bahn. 2004. 'Seasonal and spatial variation of woody tissue respiration in a Pinus cembra tree at the alpine timberline in the central Austrian Alps', *Trees*, 18.
- Yuan, Z. Y., and H. Y. Chen. 2012. 'Indirect methods produce higher estimates of fine root production and turnover rates than direct methods', *PLoS ONE*, 7: e48989.
- Yuan, Z. Y., and Han Y. H. Chen. 2010. 'Fine Root Biomass, Production, Turnover Rates, and Nutrient Contents in Boreal Forest Ecosystems in Relation to Species, Climate, Fertility, and Stand Age: Literature Review and Meta-Analyses', *Critical Reviews in Plant Sciences*, 29: 204-21.