RESEARCH PAPER

Stem and soil CO_2 efflux responses of *Pinus radiata* plantations to temperature, season, age, time (day/night) and fertilization

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Abstract

H.E. Bown, and M.S. Watt. 2016. Stem and soil CO_2 efflux responses of *Pinus radiata* plantations to temperature, season, age, time (day/night) and fertilization. Cien. Inv. Agr. 43(1):95-109. Stem CO_2 efflux is a highly variable component of the carbon budget of forest ecosystems. It reflects the balance between the CO_2 respired by the living stem tissues, less the CO_2 dissolved in the xylem sap moving upward in the transpiration stream, plus the CO_2 transported from the roots and released at the stem. Although knowledge about such fluxes at different spatial and temporal scales has markedly increased, knowledge of the effects of silviculture treatments, such as fertilization, on stem CO_2 effluxes are still limited, particularly when connecting above- and belowground processes. Using measurements obtained from twin plots (one control, one fertilized) installed in five-, 12- and 23-year-old stands of *Pinus radiata*, the objective of this study was to examine the influence of the temperature, stand age, fertilization, season and time of measurement (day/night) on the stem CO_2 efflux, soil CO_2 efflux and their ratio. There was a strong significant positive relationship between the stem CO_2 efflux and temperature. The slope between these two variables declined as the stand age increased and was higher for nighttime than daytime measurements. The stem CO_2 efflux was higher in the fertilized plots compared with the unfertilized (control) plots for the 5- and 12- but not the 23-year-old age classes. In contrast, the soil CO_2 efflux was largely unaffected by the temperature, time of measurement (day/night), fertilization and stand age; however, significantly higher values of soil CO_2 efflux were measured during spring than during the other seasons. Given the relative invariance of the soil CO_2 efflux to the temperature and treatment effects, the ratio of the stem:soil CO_2 effluxes was affected by the same factors as the stem CO_2 effluxes. These results suggest that fertilization would increase wood production and wood CO_2 efflux without changing the soil CO_2 efflux, thus most likely proportionally increasing aboveground C partitioning and decrease belowground C partitioning, with this effect being enhanced at younger ages.

Key words: Aboveground, age, belowground, CO_2 efflux, fertilization, forest plantation

Introduction

Although forests are the most important carbon (C) pool in terrestrial ecosystems, the understanding

of C flux partitioning among different components is still incomplete, with large spatial and temporal uncertainties (Litton *et al.*, 2007, Noh *et al.*, 2013). The net carbon (C) balance of forest ecosystems is the net result of CO_2 assimilation gains by photosynthesis and losses by autotrophic (plant; R_a) and heterotrophic (soil microbes and

fauna; R_h) respiration and is strongly affected by the availability of site resources, such as nutrients, water and light (Chapin *et al*., 2011). A foundational meta analysis by Litton *et al*. (2007) showed that the total above- and belowground autotrophic respiration is highly conservative at approximately 57% of gross primary productivity (GPP) independent of resource availability, competition and stand age, but C partitioning to aboveground production was strongly dependent on these factors. It is worth noting that increased nutrient availability increased the C partitioning aboveground and decreased partitioning belowground for all studies within this meta analysis.

Understanding the mechanisms that control the coupling of above- and belowground processes would provide a means to better predict ecosystem functioning (Ryan and Law, 2005). This knowledge would allow us to understand the changes that are likely to occur under different forest developmental stages, and in response to treatments, such as warming, CO_2 enrichment, nitrogen additions and irrigation, among others. Although there is considerable literature on stem CO_2 efflux (Teskey *et al*., 2008; Wieser *et al*., 2009; Brito *et al*., 2013), few studies simultaneously compare stem and soil CO_2 effluxes in response to treatments, such as fertilization, across gradients in plant age.

Pinus radiata D. Don is the most widely planted forest species in the Southern Hemisphere (Mead, 2013). Its predominance is attributable to a higher productivity, greater adaptability to soil and environmental conditions, better response to tree breeding and silviculture and a wider end-use range than most other plantation species (Turner and Lambert, 1986; Cown, 1997). Over four million hectares of plantations of this species are located mainly in the Southern Hemisphere in New Zealand, Australia, South Africa and Chile, where the species is managed under a wide range of regimes (Mead, 2013).

Fertilization is increasingly being used as a management option to increase productivity on nutrient-poor sites (Allen and Albaugh, 1999; Mead, 2013). Soil nutrient status, which is strongly linked to parent material (Turner and Lambert, 1986), is a significant determinant of growth in Australia (Boardman 1988), Chile (Toro and Gessel, 1999), New Zealand (Ross *et al*., 2009; Watt *et al*., 2010) and South Africa (Zwolinski *et al*., 2002). Overall major nutrient deficiencies in these countries include nitrogen, phosphorus, boron and zinc and less frequently magnesium, potassium, calcium, sulfur, manganese and copper (Mead, 2013). Deficiencies in nitrogen, phosphorus, magnesium and boron are common in Chilean plantations (Turner and Lambert, 1986; Toro and Gessel, 1999).

Using measurements obtained from twin plots (one control, one fertilized) installed in five-, 12- and 23-year-old stands of *P. radiata,* the objective of this study was to examine the influence of the temperature, stand age, fertilization, season and time of measurement (day/night) on stem CO_2 efflux, soil CO_2 efflux and their ratio.

Materials and methods

Study site

Measurements were taken from the Experimental Station Dr. Justo Pastor León (~300 ha) administered by the University of Chile on the western side of the Coastal Range, 25 km east of Constitución, Chile (Maule Region) (Figures 1a,b). The undulating terrain (slope 6−24°, average 14°) at the station is mostly covered by *P. radiata* with a smaller area of *Eucalyptus globulus* Labill. plantations. The natural vegetation that remains is a 'Maulino' caducifolius forest dominated by the beech tree *Nothofagus glauca* (Phil.) Krasser. The soils are Treguaco series (Inceptisol), which are developed *in situ* from metamorphic rocks, particularly micaeschists and quartz, with a sandy clay texture on the surface and clay loams at depth (CIREN, 1997). According to the Köppen-Geiger classification, the climate at the site is temperate
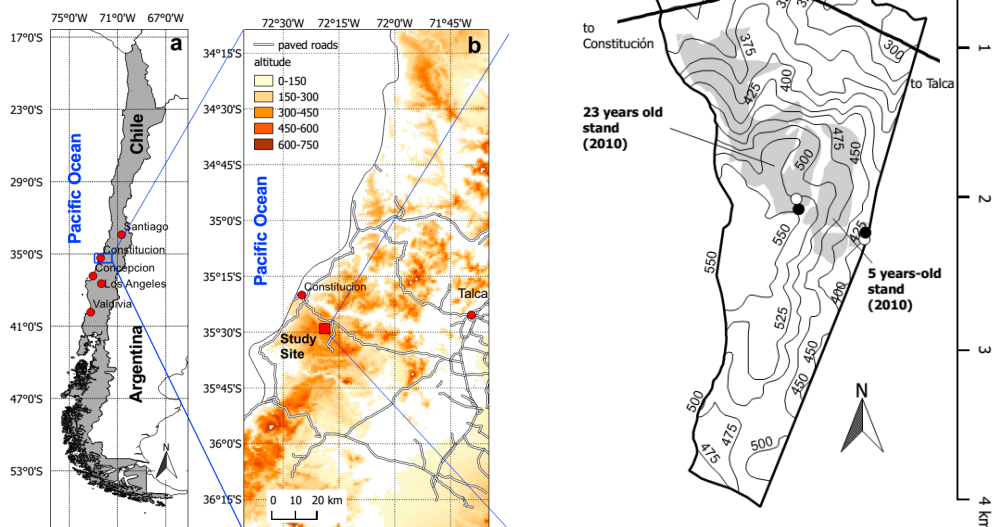
with dry and warm summers (Csb) (Peel *et al.*, 2007). The annual rainfall averages 968 mm and is very seasonal, occurring mostly (77%, 741 mm) from May (late autumn) to August (late winter). The mean annual temperature is 12.2 °C with the coldest month being July (7.6 °C) and the warmest month being January (17.1 °C) (Hijmans *et al.*, 2005).

All *P. radiata* plantations at the Experimental Station, Dr. Justo Pastor León, have a similar silviculture regime. The stands are typically established at a density of *ca.* 1250 trees ha⁻¹ (spacing 2×4 m). Following planting, chemical weed control and a preventive fertilization of diammonium phosphate (120 g per plant) and boronatrocalcite (30 g per plant) is applied. Pruning is undertaken to 50% of tree height at ages 5, 7 and 9 years. At ages 5 and 9 years, the stand densities are thinned to *ca.* 750 and 500 trees ha⁻¹, respectively. All seedlings are produced with seeds collected at the Experimental Station.

were selected in January 2010 (Figure 1c). These stands were no more than 2 km apart, and their altitude was ca. 425, 325 and 525 m above sea level, respectively. The 5- and 23-year-old stands were ca. 500 m apart in the lower and upper parts of the same east-facing slope, respectively (Figure 1c). The slope was *ca.* 25% for both stands. The 12-year-old stand was on a gentler slope (~12.5%) facing north (Figure 1c). These stands are typically second or third rotation *P. radiata* plantations established on highly eroded soils after the land was cleared to grow wheat for export during the California Gold Rush (Cousins, 2006). Despite the topographic differences among these stands, the soil profiles were similar with three distinct soil horizons (A, ~0-30 cm, silty clay loam; B, silty clay loam ~30-60 cm; C, silt loam ~60-150 cm). Crystals of michaesquists and quarts were abundant in A and B, while highly weathered rocks of these minerals were found in C. Fine (<2 mm) and medium size roots (2−5 mm) were abundant in A, decreasing sharply after 30 cm depth but still existing up to 60 cm of soil depth.

Experimental layout and treatment

Three stands within the Experimental Station aged 5- (20.4 ha), 12- (10.1 ha) and 23- (50.4 ha) years-old

Figure 1. Location of the study site in Constitución, Chile. In (c), the 5-, 12- and 23-year-old stands are shown in gray, while the twin plots are shown as open (Control) and filled (Fertilized) symbols. Contour lines at 25 m are also shown in (c).

Soil chemical analysis was carried out for a bulked sample of five randomly located 0-10 cm soil cores inside each plot. The soil C in the upper 10 cm (June 2010, before treatments were applied) ranged from 3.84−7.49% (average 5.54), while the available N and P (Olsen) were 47 mg kg^{-1} (range 21−67) and 8.1 mg kg^{-1} (range 5−12), respectively. According to Hill and Sparling (2009), these values would be marginal for N (low if N<40 mg kg^{-1}) and deficient for P (low if P<10 mg kg^{-1}). The soil B in the upper 10 cm was 0.42 (range 0.2−0.57 mg kg^{-1}) while soil-exchangeable Mg was 0.79 (range 0.34−1.33 milliequivalents 100 g^{-1}). These values are considered marginal for exchangeable Mg (low Mg <0.8 milliequivalents 100 g^{-1}) and deficient for B (low B < 1 mg kg^{-1}). Foliage chemical analysis was carried out for a bulked sample of fascicles collected in the upper third of the crown for plots of the two younger age classes and from sun-exposed fascicles in the lower third of the crown for the oldest plots before treatments were applied (May 2010). Foliar N was 1.16% (0.76−1.50% N), P was 0.13% (range 0.09−0.18), Mg was 0.16% (range 0.09−0.20) and B was 11.1 mg kg^{-1} (range 7−17). Will (1985) suggested that foliar N, P, Mg and B should be over 1.2, 0.12, 0.07% and 8 mg kg^{-1}, respectively, and therefore, our site seems to be sufficient for Mg and B but marginally deficient for N and P.

Within each stand, we established two measurement twin plots (25×25 m = 625 m^2) no more than 50 m apart with similar stand characteristics and topography (Figure 1c). Fertilizer was randomly allocated to one plot in doses equivalent to 200 kg N ha^{-1} (Urea), 46 kg ha^{-1} P (Triple Superphosphate), 10 kg ha^{-1} Mg (Sulpomag) and 2 kg ha^{-1} B (Boramat) in July 2010 and August 2011, following the stoichiometric proportions of Ingestad (1971, 1979). There was one pair of twin plots for each stand (age class); within each pair, one plot was fertilized while the other was not (control), thus totaling 6 study plots.

Measurements

Measurements of the diameter at breast height for all the trees, and height for a sample of trees (5−10), were made within each plot at the start and end of the experiment, from July 2010 and August 2011, respectively. Ten trees were randomly selected within each plot and a rubber collar was installed on each tree at heights of 0.3 m in the five-year-old plot and at 1.3 m in the 12- and 23-year-old plots. In total, there were 60 trees distributed across 6 plots throughout the fertilized and unfertilized (control) plots within the three age classes. The rubber collars (truncated cone, length 70 mm, inner cross section diameters 100 and 85 mm, volume 473 cm^3) were fixed with silicone to the tree trunk (the 85 mm cross section) so that gas leaks at the time of measurement would be minimized. Approximately 10 mm of the inner radius was covered with silicone to ensure that gas leaks were minimized so that an effective circle of 65 mm diameter was considered in the calculations. Therefore, we corrected the final measurement of CO_2 efflux by multiplying the flux by 2.36 ($(\pi (100/2)^2)/(\pi(65/2)^2)$).

The soil and stem CO_2 effluxes were measured for all ten trees in each plot using a soil respiration chamber (100 mm inner diameter, Model SRC-1, PP Systems, Herts, UK) connected to an infrared gas analyzer (Model EGM-4, PP Systems, Herts, UK). The soil CO_2 efflux was measured within 1.5 m of each tree. The stem CO_2 efflux is expressed on a stem-surface basis, while the soil CO_2 efflux is expressed on a ground-surface area basis. The soil and stem CO_2 effluxes were measured during four campaigns in spring (October 16−20, 2010), summer (March 16−20, 2011), autumn (May 16−20, 2011) and winter (August 3−7, 2011). Daytime measurements of the soil and stem CO_2 effluxes were taken between 10 a.m. and 3 p.m., while night measurements were taken between 11 p.m. and 3 a.m. Therefore, there were 480 measurements in total (6 plots × 10 trees × 4 seasons × 2 times:

day and night). The surface temperature of the trunk was taken using an infrared thermometer (Mini IR Thermometer, Model 42500, EXTECH Instruments Corporation, US) immediately after each measurement of stem CO_2 efflux. The soil temperature at 0−10 cm depth (penetration stem thermometer, Model 392050, EXTECH Instruments Corporation, USA) was taken immediately after the measurements of the soil CO_2 efflux.

During each campaign, the gravimetric water content, θ_g, was determined to a depth of 30 cm at a distance of 0.5−1.5 m from each of the ten tree trunks per plot where stem CO_2 efflux was measured. The bulk density was on average (±1 SD) 1.12±0.07 g cm^{-3} independent of plot ($F_{5,48}$=1.95, P=0.09) and soil depth (0-10 cm, 10-20 cm, 20-30 cm; $F_{2,48}$=0, P=0.99), and therefore volumetric water content, θ_v, was determined to be $\theta_v = \theta_g \times 1.12$ for all the presented results.

Data analysis

The experimental design consisted of three pairs of twin plots distributed in three contiguous *P. radiata* stands of 5-, 12- and 23-years-old. In each pair of twin plots, one plot was fertilized while the other was not. Therefore, the main effects were stand age (three levels) and fertilization (two levels). Additionally, the stem and soil CO_2 effluxes were measured repeatedly during the day and night during four seasons, yielding 48 plot averages (3 age classes × 2 fertilization treatments × 4 seasons × 2 times). Each measurement was the average of ten collars within each plot. The soil and stem temperatures and volumetric water content were considered as covariates.

All of the statistical analyses were undertaken on plot level data using SAS (SAS-Institute-Inc., 2008). Three general linear models (GLMs)

were developed and used to test the main and interactive effects of the temperature, age, time, season and fertilization on the stem CO_2 efflux, soil CO_2 efflux and their ratio. Analysis of covariance was used to test whether slopes and intercepts of the relationships between the temperature and stem CO_2 efflux, soil CO_2 efflux and their ratio significantly differed between the main or interactive effects of class level treatments (age, time, season and fertilization).

The precision of the final three developed models was assessed through the coefficient of determination (R^2), while the relative contribution of the variables was assessed through the significance level and partial R^2. The model bias was assessed through plotting residuals from each model against predicted values, class level variables and temperature. The least square means from the three final models were used to quantify how each of the class-level effects impacted the stem CO_2 efflux, soil CO_2 efflux and their ratio after corrections had been made for other effects in the model.

Climate over the course of the study

The total rainfall and mean volumetric water content (in brackets) over the course of the study for spring 2010, summer 2010/11, autumn 2011 and winter 2011 were, respectively, 118 mm (0.220), 64 mm (0.159), 467 mm (0.342) and 679 mm (0.332). The rainfall during this year (1328 mm) was much higher than the average for the 1950-2000 period (968 mm) (Hijmans *et al.*, 2005). Most of the rainfall at the site occurred during the winter months (Figure 2b). The air temperature showed a strongly seasonal pattern over the study period (Figure 2a). The average daily temperature was 11.0 °C (range 1.6−30.8 °C), the average daily minimum was 4.7 °C (range −4.0−14.9 °C), and the average daily maximum was 18.2 °C (range 5.7−33.2 °C).
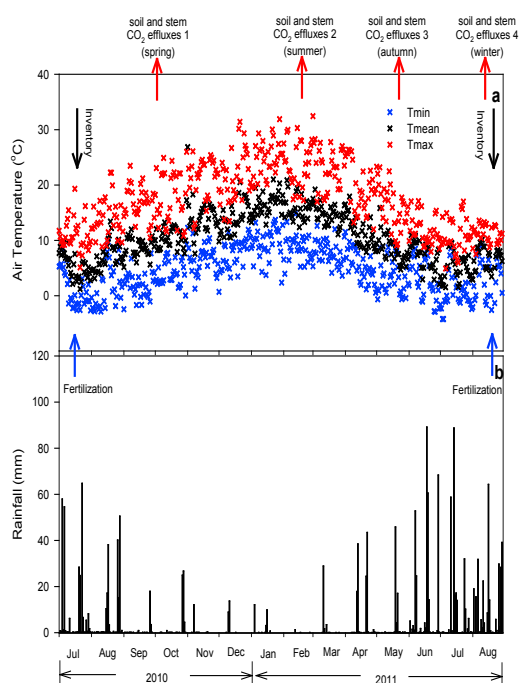
Figure 2. (a) Minimum (blue crosses), mean (black crosses) and maximum (red crosses) air temperature and (b) daily rainfall for the study site during the measurement period. The timings of the stem and soil CO_2 efflux measurements and fertilization that were carried out are shown in the figure.

Results

Variations in stand characteristics

The stand density was lower for the 23-year-old stand than for either the five- and 12-year-old stands, as these older plots had received an additional thinning (Table 1). The initial tree characteristics were relatively similar between the fertilized and the unfertilized plot within each age class for both the DBH and height (Table 1). However, the initial basal area for the fertilized five-year-old plot was lower than that of the unfertilized plot as DBH, height and stand density in the fertilized plot were lower than that of the unfertilized plot at the onset of the experiment.

Within each age class there was generally a positive response to fertilization. The annual growth gains for the DBH were greater in the fertilized than unfertilized treatments for all age classes, while the height growth was relatively independent of fertilization. The gains from fertilization over the study period were relatively greater for

Table 1. Treatment variation in the mean tree dimensions and CO_2 effluxes from the soil and stem. For the diameter at breast height (DBH), height and basal area, the values show the mean at the start of the experiment followed by the percentage change in growth, in brackets, over the course of the measurement year. Stand density did not change throughout the experiment.   For CO_2 effluxes, the mean values averaged over two measurement times (night, day) and four seasons are shown. As age classes and the fertilization treatment were not replicated, descriptive rather than inferential statistics are presented.

Age (yrs)	Treatment	Stand characteristics				Respiration (μmol CO_2 m^{-2} s^{-1})	
		DBH (cm)	Height (m)	Basal area (m² ha⁻¹)	Stand density (stems ha⁻¹)	Soil	Stem
5	Control	8.5 (24%)	5.8 (40%)	5.2 (55%)	912	4.19	8.03
5	Fert.	7.3 (39%)	5.0 (38%)	3.1 (94%)	736	3.98	8.67
12	Control	14.5 (8%)	13.4 (12%)	15.6 (17%)	944	4.16	4.17
12	Fert.	15.2 (12%)	14.6 (9%)	16.3 (25%)	896	4.26	6.00
23	Control	30.9 (1%)	30.8 (1%)	38.5 (2%)	512	3.79	1.09
23	Fert.	29.4 (3%)	28.2 (2%)	36.9 (6%)	544	5.06	1.01

the basal area, than either the height or DBH and were substantial for the five-year-old (94 vs. 55%), moderate for the 12-year-old (25 vs. 17%), and negligible for the 23-year-old (6 vs. 2%) age classes (Table 1). As there was no change in the stand density throughout the course of the year, these gains were entirely attributable to changes in the tree diameter.

At the plot level, the stem CO_2 efflux averaged 4.83 µmol CO_2 m^{-2} (stem surface) s^{-1} with a range of 0.344 – 13.7 µmol CO_2 m^{-2} s^{-1}. Variation with age was most marked, with values increasing from 1.01 µmol CO_2 m^{-2} s^{-1} in the 23 year-old fertilized plot to 8.67 µmol CO_2 m^{-2} s^{-1} for the 5 year-old fertilized plot (Table 1). The soil CO_2 efflux averaged 4.24 µmol CO_2 m^{-2} s^{-1} with a range of 2.06 – 8.44 µmol CO_2 m^{-2} s^{-1}. Little systematic variation in the soil CO_2 efflux was evident across the age range (Table 1). The ratio of the stem : soil CO_2 efflux averaged 1.39 with a range of 0.102 – 4.05. Similar to the stem CO_2 efflux, the ratio declined with increasing stand age from 2.77 to 0.25, respectively, for five- and 23-year-old stands that were fertilized.

Stem CO_2 efflux

The analysis of variance showed that the stem CO_2 efflux was significantly affected by the interactions of stem temperature × time × age and fertilization × age (Table 2). An examination of the partial R^2 values showed the respective values of 0.86 and 0.06 for stem temperature × time × age and fertilization × age, denoting the importance of the three-way interaction (Table 2).

The positive linear relationships between the stem temperature and stem CO_2 efflux for the six combinations of time and age exhibited a strong pattern, with model predictions shown as lines in Figure 3a. The predictions of the stem CO_2 efflux (lines in Figure 3a) generally fitted the measurements (symbols in Figure 3a) well for the six time × age combinations. The slope between the stem temperature and stem CO_2 efflux, shown in Figure 3b, exponentially declined with increasing stand age for both the night (closed circles, Figure 3b) and daytime (open circles, Figure 3b) measurements. The slope for measurements taken during the night was markedly higher than the slope of daytime measurements for the five year-old stands (0.788 vs. 0.477 µmol CO_2 m^{-2} s^{-1} ^{o}C^{-1}), but these slopes converged as the stand age increased to a minimum difference in the 23-year-old age class (0.175 vs. 0.127 µmol CO_2 m^{-2} s^{-1} ^{o}C^{-1}) (Figure 3b).

The age × fertilization interaction was significant with least square means showing fertilization to be associated with increases in the stem CO_2 efflux for the five- and 12-year-old stands (Figure 4a) that were marginally insignificant (P=0.0528) at five years of age (9.43 vs. 8.22 µmol CO_2 m^{-2} s^{-1}) and highly significant (P=0.0009) at 12 years (6.11 vs. 3.91 µmol CO_2 m^{-2} s^{-1}). For the 23-year-

Table 2. Regression showing the influence of temperature (stem surface temperature for stem CO_2 efflux, soil temperature for soil CO_2 efflux and the average of the two for the ratio of stem:soil CO_2 efflux) and treatment effects on the stem and soil CO_2 effluxes and the ratio of stem: soil CO_2 effluxes. For the test of significance (Sig.), the F values followed by the P categories are shown. Asterisks ***, **, * denote significance at P=0.001, 0.01 and 0.05, respectively. Values for insignificant terms (P>0.05) have been omitted for clarity. The partial and cumulative (in brackets) coefficient of determination (R^2) is also shown for all significant terms in each model. Temperature was included in the model as a continuous variable, while all other terms were included as categorical variables.

Term	Stem CO_2 efflux		Soil CO_2 efflux		Ratio stem: soil CO_2 efflux	
	Sig.	R^2	Sig.	R^2	Sig.	R^2
Age × Fert.	4.91**	0.06 (0.06)	2.93*	0.11 (0.11)	2.70*	0.05 (0.05)
Age × Season			4.02**	0.47 (0.58)		
Temp × Age × Time	16.5***	0.86 (0.92)			7.22***	0.83 (0.88)
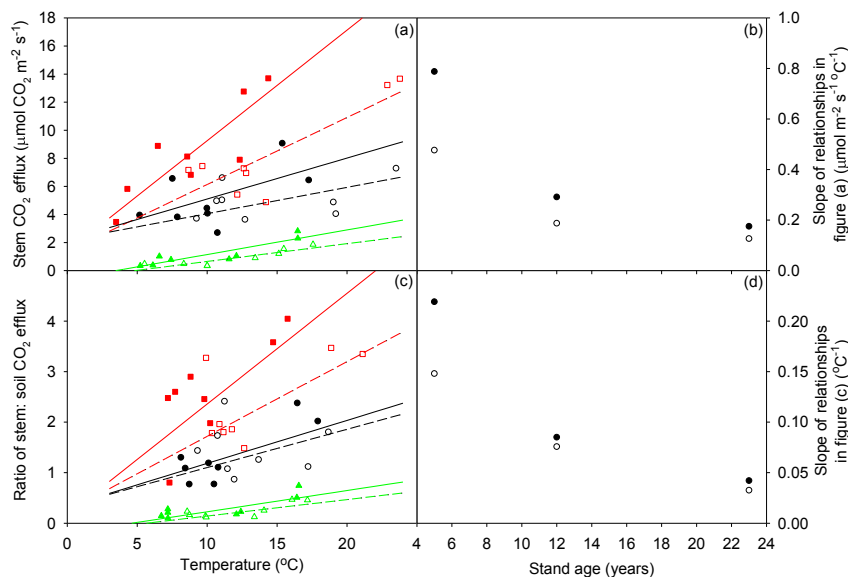
Figure 3. Relationship between temperature and (a) stem CO_2 efflux and (c) ratio of stem:soil CO_2 efflux for five (red squares), 12 (black circles) and 23 (green triangles) year old stands measured during the night (filled symbols) and day (open symbols). Using model parameters lines are fitted to five (red lines), 12 (black lines) and 23 (green lines) year old stands measured during the night (solid lines) and day (dashed lines). Stem CO_2 efflux is regressed against stem temperature while the ratio of stem:soil CO_2 effluxes is regressed against the mean of stem and soil temperature. Panels (b) and (d) show the slopes of the lines in panels (a) and (c), respectively, plotted against stand age for measurements taken during the night (filled circles) and day (open circles). Each point in (a) and (c) is the average of measurements of 10 trees.

old stands, the values of the stem CO_2 efflux in the control exceeded those of the fertilized treatment, but the differences were very small (1.21 vs. 1.13 µmol CO_2 m^{-2} s^{-1}) and insignificant (P=0.902) (Figure 4a).
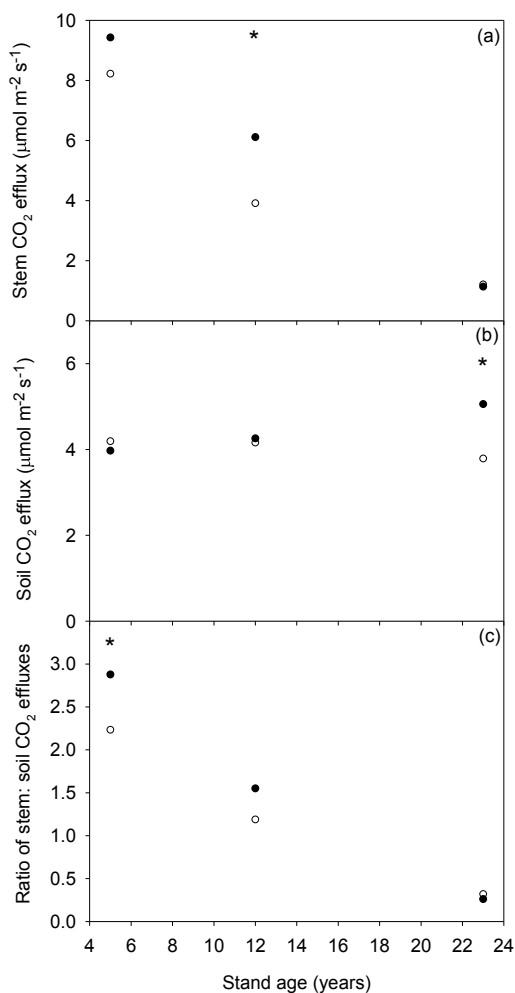
Soil CO_2 efflux

The final model accounted for 58% of the variance in the soil CO_2 efflux (Table 2). Of the two terms included in the model, the age × season effect contributed far more than the age × fertilization interaction (partial R^2 of 0.47 vs. 0.11, Table 2). Neither the age nor the soil temperature significantly (at P=0.05) affected the soil CO_2 efflux (Figure 5).

The interaction between age and season was primarily attributable to high spring soil CO_2

efflux values in the 23-year-old age class (6.44 µmol CO_2 m^{-2} s^{-1}) that significantly (P range of 0.01 − 0.0001) exceeded values for all other three seasons within the 23-year-old age class and values for all four seasons within both the five- and 12-year-old age classes (see the green crosses in Figure 5).

There was a significant interaction between fertilization and age class (Figure 4b) with values of the soil CO_2 efflux in the 23-year-old fertilized plots, significantly (P=0.0069) exceeding that of the unfertilized plots by 34% (5.06 vs. 3.79 µmol CO_2 m^{-2} s^{-1}). The effects of fertilization on the soil CO_2 efflux in the younger age classes were not significant, and the response direction varied (Figure 4b). Fertilization was associated with small insignificant (P=0.824) increases in the soil CO_2 efflux within the 12-year-old age class (4.26 vs. 4.16 µmol CO_2 m^{-2} s^{-1}) and small insignificant
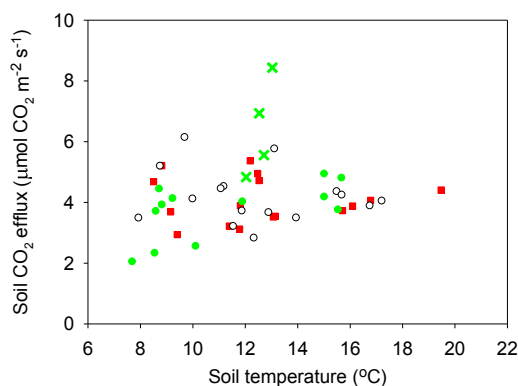
Figure 4. Relationship between stand age and (a) stem CO_2 efflux (b) soil CO_2 efflux and (c) the ratio of stem: soil CO_2 efflux for fertilized (filled circles) and unfertilized plots (open circles). Values are least square means from the three models with asterisks denoting treatment differences at P=0.05.

Figure 5. Relationship between soil CO_2 efflux and soil temperature in five year old stands measured during the spring (green crosses) and summer, autumn and winter (green filled circles). Also shown are measurements taken in 12 (black open circles) and 23 (red filled squares) year old stands.

(Table 2). The examination of the partial R^2 values for the terms in the model showed that temperature × time × age had the strongest influence on the ratio with a value of 0.83, while fertilization × age had a lower partial R^2 of 0.05 (Table 2).

The temperature × time × age interaction indicated that the slopes in the relationship between temperature and the ratio were significantly different between the six time × age combinations. The examination of these relationships in Figure 3c showed that the model fitted the data well and illustrates the systematic change in the slopes with respect to time and age. The slope declined exponentially with increasing stand age (Figure 3d). Although slopes for night CO_2 effluxes were consistently higher in all age classes (Figure 3d), the differences in the slope were most marked for the youngest age class (0.219 vs. 0.148 $^{\circ}C^{-1}$), with the differences diminishing greatly for the 12- (0.0850 vs. 0.0757 $^{\circ}C^{-1}$) and the 23-year-old age classes (0.0420 vs. 0.0326 $^{\circ}C^{-1}$).

The significant age × fertilization interaction was attributable to variation in the effect of fertilization on the ratio across the three age classes (Figure 4c). The least square means show that the plots in which fertilizer had been applied had signifi-

(P=0.615) reductions in the five-year-old age class (3.93 vs. 4.19 µmol CO_2 m^{-2} s^{-1}).

Ratio of stem to soil CO_2 effluxes

The final model, which incorporated the interactions of the temperature (averaged between the soil and stem) × time × age and fertilization × age accounted for 88% of the variation in the ratio

cantly (P=0.0053) higher values for the ratio in the 5-year-old age class compared with the other age classes (2.88 vs. 2.23), but this difference declined and was insignificant at age 12 (P=0.11, 1.55 vs. 1.19). The difference was also insignificant at age 23 (P=0.778), and the unfertilized plot had higher ratio values than the fertilized plot within this age class (0.319 vs. 0.260).

Discussion

Increased stem CO_2 efflux in response to fertilization has previously been reported for *P. radiata* (Ryan *et al.*, 1996), *Picea abies* (Stockfors and Linder, 1998) and *P. taeda* (Maier 2001). Fertilization also increased the dark respiration rates of current and 1-year-old shoots of *Pseudotsuga menziesii* (Brix, 1971). The increases in stem CO_2 efflux in response to fertilization are likely to be attributable to increases in growth, the amount of living tissues, protein concentration and turnover (Stockfors and Linder, 1998). Therefore, the lack of a stem CO_2 efflux response at age 23 in our study is most likely attributable to the negligible growth response to fertilization observed at this age.

Increased nutrient supply has been reported to shift the allocation of carbon from belowground to aboveground processes (Litton *et al*., 2007), and we found that early fertilization did increase the stem CO_2 efflux and wood production while maintaining relatively constant soil CO_2 efflux. Giardina *et al.* (2003) showed that fertilization-related increases in the gross-primary productivity (GPP) in *E. saligna* plantations in Hawaii were entirely allocated aboveground, while the total belowground carbon flux (TBCF) did not change with fertilization. Our results conform to those of Giardina *et al.* (2003).

Because autotrophic and heterotrophic activity belowground are controlled by substrate availability, soil CO_2 efflux is expected to be strongly linked to plant metabolism, photosynthesis and litterfall (Ryan and Law, 2005). In fact, Litton

et al. (2007) found strong linear relationships between both aboveground net primary productivity (ANPP) and GPP, and TBCF-GPP at the scale of years. However, our 480 instantaneous paired measurements of the soil and stem CO_2 effluxes were uncorrelated (R^2<0.01, P=0.45), and we additionally found that the ratio of the stem to soil CO_2 effluxes increased with the temperature and changed for the six time (day/night) × age combinations and with the fertilization treatments. Therefore, stable long-term C partitioning ratios for the above- and belowground primary production may break down when examined over shorter time scales for *P. radiata*.

We found that the stem CO_2 efflux was significantly greater during the night than during the day, as has also been found in other studies (Lavigne *et al*., 1996, Gruber *et al*., 2009). A lag in the respiration response to the temperature (Ryan *et al*., 1995) and the dissolved CO_2 moving upward in the transpiration stream during the day (Maier and Clinton, 2006) have been given as likely explanations for this phenomena. The delayed responses of the stem respiration to temperature may be the result of a large resistance to the movement of the CO_2 from the stem into the air (Lavigne *et al*., 1996), which would require a large increase in the internal CO_2 concentration before an increased rate of diffusion from the stem could occur. As the [CO_2] inside the stems might be 50−250 (2-10%) times greater than the concentration in the ambient air (0.04%) (Maier and Clinton, 2006; Teskey *et al*., 2008), a large concentration gradient must exist to drive the CO_2 diffusion given a large stem-CO_2 diffusion resistance. If the volume of the air inside the stems is large relative to the diffusion rate, then it would take time for the internal CO_2 concentration to increase sufficiently to drive CO_2 diffusion at a higher rate when the temperature rises.

The second possible explanation is that some CO_2 produced by the respiration of the stem, might dissolve in the xylem sap as it moves upward in the transpiration stream and be respired or assimilated

by the leaves (Maier and Clinton, 2006; McGuire *et al*., 2009). Because most transpiration occurs during the day, the stem CO_2 efflux is expected to be lower during the day than at night (Martin *et al*., 1994; Maier and Clinton, 2006; Teskey *et al*., 2008; Höltta and Kolari, 2009: Gruber *et al*., 2009; Brito *et al.,* 2010). Alternative explanations include the possibility that (i) the temperatures measured a short distance beneath or at the surface of the bark, as in this study, may not be representative of the temperatures experienced by most of the respiring biomass (Derby and Gates, 1966); (ii) some CO_2 is stored temporarily (Höltta and Kolari, 2009); and (iii) stem respiration depends also on plant water stress (Lavigne, 1987; Teskey *et al*., 2008; Saveyn *et al*., 2007), which might be more severe during the day than during the night. Independent of these reasons, the extrapolation of daytime measurements over the course of the night might underestimate the nighttime respiration rates by 18−40% (Lavigne *et al*., 1996). We found this underestimation could be even greater in our study (e.g., 31−57% at 15 °C, see Figure 3a).

Some CO_2 from root and microbial respiration could also move upward dissolved in water and be released at the stem (Maier and Clinton, 2006; Teskey and McGuire, 2007; Teskey *et al*., 2008). Moore *et al.* (2008) estimated that soils could contribute as much as 19% of the average stem CO_2 efflux. Kodama *et al.* (2008) found that the C isotope signatures of the soil and stem CO_2 effluxes were uncorrelated, suggesting a small to nonexistent contribution of soil CO_2 to the stem CO_2 efflux in *P. sylvestris*. The soil CO_2 efflux in our study was relatively insensitive to different environmental drivers, including the soil temperature, although seasonal variations with a peak in spring were observed. Giardina and Ryan (2002) found similar responses of the soil CO_2 efflux with a peak in summer in *Eucalyptus* plantations in Hawaii. As the stem CO_2 efflux was highly sensitive to the temperature, age, time (day/night) and fertilization, while the soil CO_2 efflux was not, we speculate that even if soils made a

19% contribution (Moore *et al*., 2008) to the stem CO_2 efflux, that amount is not large enough to change the conclusions of our study.

The stem CO_2 efflux markedly declined with increasing stand age in our study, as found previously for jack pine (Lavigne and Ryan, 1997; Ryan *et al*., 1997) and *E. saligna* (Ryan *et al*., 2004). This age-related decline in stem respiration has been attributed to decreasing growth, the sapwood area or living cells, and the protein concentration and turnover as trees age (Ryan *et al*., 1997). Living stem cells are found in the inner bark, vascular cambium and ray cells in the sapwood, with the latter decreasing in proportion with tree size (Teskey *et al*., 2008) and, hence, probably age. Carey *et al.* (1997) showed that maintenance respiration per unit of sapwood volume decreased in ponderosa pine with increasing tree diameter in both desert and montane ecosystems. Such a decrease in the stem maintenance respiration with age (or tree diameter) was most likely due to fewer live cells per unit volume of wood, the decreasing Q_{10} with tree diameter, or the increased resistance to diffusion of CO_2 across a greater sapwood width in larger trees. Ryan *et al.* (1996) found that among woody components, the branches in the upper canopy and the small diameter coarse roots had the highest respiration rates compared with other tissues, providing further evidence that stem CO_2 efflux depends on size and age. The thickening of the bark as a tree ages may also well reduce CO_2 diffusion to the atmosphere (Teskey *et al*., 2008). In contrast, soil CO_2 efflux was relatively insensitive to plantation age in our study, which was consistent with the findings from *Eucalyptus saligna* in Hawaii (Giardina and Ryan, 2002).

The observed differences in the stem and soil CO_2 efflux among the stands could have been caused by differences in the sites, site management and stand density, rendering invalid this space-for-time substitution. In terms of the site differences, the stands were no more than 2 km apart, and although there were some differences in aspect, the soil and chemical properties of the sites

were very similar. Litton *et al.* (2007), in a meta analysis of 63 studies, showed that intraspecific competition (stand density) did not have a large or consistent effect on C partitioning. Similarly, Noh *et al.* (2013) found that C partitioning belowground did not change between two mature stands of *Pinus densiflora* with contrasting stand densities, although the absolute amounts of the aboveground net primary productivity and total belowground carbon fluxes were greater at higher densities. Therefore, we believe that the overall responses of the stem and soil CO_2 effluxes and their ratio to temperature, age, time (day/night) and fertilization were not confounded by the stand density or management regime.

The experimental design for the study exhibited some limitations, such as no replication, small treatment plots, a single site and an experimental time of only one year. Despite the limitations, repeated measurements were carried out for four seasons, during the day and night, for six plots distributed in three age classes and one fertilization treatment (fertilized, unfertilized), which made it feasible to fit general linear models (GLMs) to test the main and interactive effects of the temperature, age, time, season and fertilization on stem CO_2 efflux, soil CO_2 efflux and their ratio. Despite the validity of the statistical models used, our results need to be considered with caution as they were drawn from one site, and results for other sites could change compared with our study.

In conclusion, the stem CO_2 efflux increased with the temperature of the woody tissue, as many other studies have found (Teskey *et al.*, 2008; Gruber *et al.*, 2009; Brito *et al.*, 2010, 2013; Yang *et al.*, 2012). Thus, the stem CO_2 efflux increased according to the following series: winter, autumn, spring and summer. The slopes between the temperature and stem CO_2 efflux were found to decline with stand age and were higher for measurements made during the night than those taken during daytime. The soil CO_2 efflux was relatively insensitive to the soil temperature, age, time of measurement (day/night) and fertilization. However, the soil CO_2 efflux was generally greater in spring compared with other seasons. The soil and stem CO_2 effluxes were uncorrelated. Early fertilization markedly increased wood growth and the wood CO_2 efflux without significantly increasing the soil CO_2 efflux. From a silviculture and management perspective, our results suggest that early fertilization would substantially increase stem growth and stem CO_2 efflux, without changing the soil CO_2 efflux, with this effect being reduced as plantations age.

Acknowledgements

Resumen

H.E. Bown, y M.S. Watt. 2016. Respuesta de los flujos de CO$_2$ de los fustes y de los suelos a la temperatura, estación del año, edad, tiempo (día/noche) y fertilización en plantaciones de *Pinus radiata*. Cien. Inv. Agr. 43(1):95-109. El flujo de CO$_2$ desde los fustes es una componente altamente variable en el balance global de carbono en ecosistemas forestales. Refleja el balance entre el CO$_2$ respirado por los tejidos vivos del fuste, menos el CO$_2$ disuelto en la savia del xilema que se mueve hacia arriba como resultado de la transpiración, más el CO$_2$ transportado desde las raíces y liberado en el fuste. Aunque el conocimiento acerca de estos flujos a diferentes escalas espaciales y temporales ha aumentado marcadamente, los efectos de tratamientos silviculturales, tales como la fertilización, en la respiración de los fustes es aún limitada particularmente al conectar procesos arriba y abajo del suelo. Usando medidas en parcelas gemelas (una parcela testigo, una parcela fertilizada) instaladas en rodales de 5,12 y 23 años de edad de *Pinus radiata,* el objetivo del estudio fue examinar la influencia de la temperatura, edad, fertilización, temporada y tiempo del día (día/noche) en los flujos de CO$_2$ desde los fustes, desde los suelos y la razón entre ellos. Se encontró una fuerte relación positiva entre el flujo de CO$_2$ desde los fustes con la temperatura de los mismos. La pendiente entre estas dos variables declinó con la edad, siendo superior durante la noche que durante el día. El flujo de CO$_2$ desde los fustes fue mayor en las parcelas fertilizadas que en las testigo en los rodales de 5 y 12 años pero no en el de 23 años. En contraste, los flujos de CO$_2$ desde los suelos fueron mayoritariamente invariantes a la temperatura del suelo, al tiempo de medición (día/noche), fertilización y edad de los rodales; aunque dichos valores fueron significativamente mayores durante la primavera comparado con las otras temporadas. Dada la relativa invariabilidad de los flujos de CO$_2$ de los suelos a la temperatura y a los efectos de los tratamientos (fertilización, edad, día/noche), la razón de los flujos de CO$_2$ entre fustes y suelos fueron afectados por los mismos factores que afectaron los flujos de CO$_2$ desde los fustes. Estos resultados sugieren que la fertilización temprana aumenta drásticamente la producción y flujos de CO$_2$ de los fustes, sin cambiar los flujos de CO$_2$ desde los suelos; o en otras palabras, asignando proporcionalmente más carbono arriba del suelo y menos abajo del suelo, con este efecto decreciendo con la edad de la plantación.

Palabras clave: Arriba del suelo, bajo el suelo, edad, fertilización, flujos de CO$_2$, plantación forestal.

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