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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

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Summary

• Root respiration at the level of a forest stand, an important component of ecosystem carbon balance, has been estimated in the past using various methods, most of them being indirect and relying on soil respiration measurements.

• On a 3-yr-old *Eucalyptus* stand in Congo-Brazzaville, a method involving the upscaling of direct measurements made on roots *in situ*, was compared with an independent approach using soil respiration measurements conducted on control and trenched plots (i.e. without living roots). The first estimation was based on the knowledge of root-diameter distribution and on a relationship between root diameter and specific respiration rates.

• The direct technique involving the upscaling of direct measurements on roots resulted in an estimation of 1.53 μmol m^{-2} s^{-1}, *c.* 50% higher than the mean estimation obtained with the indirect technique (1.05 μmol m^{-2} s^{-1}).

• Monte-Carlo simulations showed that the results carried high uncertainty, but this uncertainty was no higher for the direct method than for the trenched-plot method. The reduction of the uncertainties on upscaled results requires more extensive knowledge of temperature sensitivity and more confidence and precision on the respiration rates and biomasses of fine roots.

Key words: decomposition, *Eucalyptus*, soil respiration, stand-level root respiration, uncertainty analysis, up scaling.

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Introduction

The carbon (C) balance of a forest stand and its response to management practices and changing environmental conditions has been placed in the scientific limelight in the context of the current search for C sinks. Fast-growing tropical planted forests are now arousing particular interest in this respect because of their economic interest for 'Clean Development Mechanisms' and their potentially high C sink performances (Glenday, 2006; Yong Shin *et al.*, 2007). Certain species of the *Eucalyptus* genus have been extensively planted and managed for pulpwood production in many tropical regions, because they are among the most productive and adaptable species available and can be managed profitably on short

rotations (5–7 yr). Ecosystem models capable of simulating water, C and nutrient fluxes are continually being developed and improved in order to assess their C balance and response to driving environmental variables (Battaglia *et al.*, 2004; Santos & Costa, 2004; Corbeels *et al.*, 2005; Miehle *et al.*, 2006). One of the challenges involved in the experimental validation of these process-based models is the separation of the soil CO_2 efflux (R_s) into its heterotrophic (R_h, respiration of microbes decomposing litter and soil organic C) and 'autotrophic' (R_{ar}, respiration of root and associated rhizospheric microbes) components (Epron *et al.*, 2001; Bond-Lamberty *et al.*, 2004b). The complexity of the interactions between the rhizosphere and the surrounding soil (physical environment, priming and/or competition effects (Subke *et al.*, 2004; Kuzyakov & Larionova, 2006)) makes the experimental determination of the different components extremely difficult, as any manipulation of the system in view of separation will necessarily disrupt these interactions. This partitioning is, however, important: although R_h and R_{ar} are linked because of the interaction of their sources below ground, the first intervenes in the C balance of the soil and the second in that of the tree, which most models treat separately. The determination of the proportion between R_{ar} and R_h must therefore be attempted with as much precision and accuracy as possible, and has been the object of a number of studies, using a variety of different techniques, each with their own drawbacks and underlying assumptions (see reviews by Hanson *et al.*, 2000; Subke *et al.,* 2006). Some common techniques involve comparing soil respiration measurements on normal and root-free plots: roots are killed by digging of a trench around the plot (Epron *et al.*, 1999), or deprived of their supply of photosynthates by girdling of the tree (Hogberg *et al.*, 2002). The root component is then obtained by subtracting R_h (supposedly measured on the root-free plots) from R_s and applying a number of corrections. A disadvantage of these techniques is that the estimations of the component fluxes are bound to be correlated, as one is deduced from the other. A natural correlation is expected because of the intricate links between soil organic C and the rhizosphere, but an extra artificial correlation may be added because of experimentation (Bond-Lamberty *et al.*, 2004b). In addition, these techniques rely heavily on soil respiration measurements, whose accuracy and precision are an ongoing matter of debate (Janssens *et al.*, 2000, 2001; Longdoz *et al.*, 2000; Rayment & Jarvis, 2000; Yim *et al.*, 2002; Ngao *et al.*, 2006). In order to validate estimates of root and heterotrophic respiration, all possible approaches allowing the independent estimation of the two components must be explored.

Direct gas-exchange measurements on living roots and subsequent upscaling are one such option for the independent estimation of the autotrophic component. This is a standard approach for the quantification of above-ground woody respiration (Ryan *et al.*, 1996; Clinton & Vose, 1999; Ceschia *et al.*, 2002; Damesin *et al.*, 2002; Vose & Ryan, 2002; Bolstad *et al.*, 2004). However, the rare examples of such studies on roots (Tate *et al.*, 1993; Ryan *et al.*, 1996; Uchida *et al.*, 1998) suffered from small samples and employed quite a simple upscaling technique based on the multiplication of root biomass by respiration rates. Yet, several studies that have addressed the question of the quantification of root respiration at the organ level have shown that CO_2 efflux rates are highly variable from one root to another. Different factors influencing respiration rates have been proposed, including temperature (Atkin *et al.*, 2000; Bond-Lamberty *et al.*, 2004a), root diameter (Pregitzer *et al.*, 1998; Desrochers *et al.*, 2002), nitrogen (N) and total nonstructural C content (Ryan *et al.*, 1996; Zogg *et al.*, 1996; Burton *et al.*, 2002; Desrochers *et al.*, 2002), root function and depth (Pregitzer *et al.*, 1998) and season (related to above-ground growth) (Desrochers *et al.*, 2002; Misson *et al.*, 2006). The incorrect accounting for the variability of the structure and respiration rates of the root system can potentially lead to large errors in upscaled estimates of the root contribution to the tree's C balance. A more precise estimation of stand-level root respiration would therefore require either huge samples or, ideally, the use of a scaling-up variable and of a known relationship between root respiration and this variable. This method has so far not been applied, as it requires extensive knowledge of the root system, which is very difficult and time-consuming to obtain.

Our study provides a first attempt at the use of this upscaling technique to quantify the stand-level root respiration flux, using root diameter as the scaling-up variable. Direct *in situ* root respiration measurements were carried out on fine and coarse roots of a 3-yr-old *Eucalyptus* stand in southern Congo and, thanks to a large number of samples, a strong relationship was established between root diameter and CO_2 efflux rate. Using a detailed study of the architecture of the root system we were able to scale these results up to the stand level in order to obtain a first estimation of R_{ar}. An independent estimate was obtained by the more traditional trenched-plot technique on the same stand and over the same period. The two estimations are compared and the uncertainties associated with both methods are discussed in detail, aiming to identify key factors for improvement of the precision of the estimates.

Materials and Methods

Study site

The study site is located in the Atlantic coastal zone of the Republic of Congo-Brazzaville, in central Africa. Large *Eucalyptus* plantations (some 40 000 ha) have been managed for pulpwood production around the city of Pointe Noire (4°S, 12°E, 100 m elevation) for *c.* 30 yr. The original vegetation was a savannah dominated by the C4 Poaceae *Loudetia arundinacea*. Mean annual air humidity and temperature are high (85% and 25°C, respectively), with low seasonal variations (2% and 5°C, respectively). Annual precipitation

averaged 1400 mm between 1998 and 2003, with a dry season between May and September, which is marked by uniform cloudy conditions and cooler temperatures.

The deep sandy soils are classified as ferralic arenosols according to the FAO (Food and Agriculture Organization) classification (Trouvé *et al.*, 1994), with high sand (80–90%) and low clay (8–10%) and silt (2–2.5%) contents. These soils are characterized by low water retention, a very low level of organic matter (the top 5 cm of mineral soil contain from 0.45 to 1.1 % of C, with a mean C content of 0.77 % for a 3-yr-old stand (Trouvé *et al.*, 1994)) and a poor cationic exchange capacity (Nzila *et al.*, 2002).

The site considered in this study is an industrial stand, first afforested with *Eucalyptus* of the EP-PF1 clone in 1981, after destruction of the native savannah by harrowing. After the first harvest in 1988, two subsequent coppices were conducted and harvested in 1995 and 2002 (selection of one or two rejects on each stump 6 months following the harvest). After each harvest, all branch, leaf and bark residues were left on site. One year following harvest each coppice received an application of NPK 13–13–21 fertilizer (200 kg ha^{-1}). In 2002 the stand was subsoiled in the plantation row and replanted with a fast-growing clone of the hybrid *Eucalyptus urophylla* × *Eucalyptus grandis* (currently the most commonly planted clone in Congo) at a density of 750 trees ha^{-1}. Each tree received an initial dose of NH_4NO_3 fertilizer of 200 g per plant and regular chemical weeding was carried out using glyphosate (3 l ha^{-1}).

No specific study of root mycorrhization was undertaken, but a native ectomycorrhiza identified as *Scleroderma* sp. (Garbaye *et al.*, 1988) is commonly found in these Congolese stands.

Roots extend very far down in this sandy profile: augur sampling detected fine roots down to a depth of > 9 m in a similar nearby stand (Laclau *et al.*, 2001). Nevertheless, all studies in neighboring *Eucalyptus* stands show that fine-root density (measured in number of root impacts per unit area on vertical profiles) decreases rapidly down to a depth of 30 cm, then remains stable at least to a depth of 2 m (Laclau *et al.*, 2001; Bouillet *et al.*, 2002). On our particular stand, soil core measurements of fine roots (< 2 mm diameter) revealed that 46% of all fine root biomass measured down to a depth of 1.2 m was located in the top 30 cm of soil and the litter layer. Litter accumulation starts at the end of the first year after planting (Nzila *et al.*, 2002) and leads to a continuous accumulation in the forest floor and a slight enrichment of organic C in the first 10 cm of soil (however, the depth of this organic horizon is still very limited at the age of 3 yr).

Estimation of autotrophic and heterotrophic components of soil CO_2 efflux using soil respiration measurements

Soil respiration measurements were made every 2 wk on control and trenched-plots from February 2005 to October 2005, using a dynamic closed-path Li8100 system equipped with a 10 cm diameter Li8100 102 respiration chamber (LiCor Inc., Lincoln, NE, USA). Plots were composed of nine PVC collars placed on a square grid surrounding a central tree. The grid was designed to represent the area less than half-way between the central tree and the surrounding trees. In January 2005 17 plots were created and their CO_2 efflux at the soil surface was measured. Six plots were selected for the rest of the study. Three of these plots were kept undisturbed as control plots. In March a 2-m deep trench was dug around each of the other three plots, and lined with heavy plastic film to block the growth of new roots into the plot. The central tree was felled and its stump was treated with glyphosate, thus ensuring that all roots in the trenched-plot were killed. The mean respiration before trenching of the three trenched plots and that of the three control plots was similar to the mean efflux of the 17 original plots.

Three blocks were formed, each including one trenched-plot and one control plot whose soil CO_2 efflux before trenching were similar. These blocks were used as a basis for the subsequent upscaling of root respiration estimations.

Soil respiration was measured twice-monthly on all plots starting in February 2005, but trenched-plot measurements were only considered reliable after day 120 (allowing a certain period for the severed roots to die). Soil volumetric water content in the 0–6 cm depth (θ) was measured simultaneously within 5 cm of the collars using a Theta Probe (ML2, Delta-T Device Ltd, Cambridge, UK). In June 2005 daily soil water content measurements were also carried out using the same Theta Probe, and soil temperatures were measured every half hour. Temperature was measured with copper-constantan thermocouples inserted along a profile at five different depths (1, 2, 4, 8 and 30 cm). The difference between depths was small (no doubt owing to the uniform cloudy conditions prevailing at that time of the year). The average absolute temperature difference between the surface soil and the deepest layer being only 1.2°C, we calculated the soil temperature as the average over the profile.

Soil CO_2 efflux of each block was estimated as the average of the nine measurements of each treatment. The average of the trenched-plot measurements was used for the estimation of heterotrophic respiration, after application of a correction in order to take into account the extra CO_2 flux resulting from the decomposition of the killed root system.

Temporal evolution of the mass of the remaining root system in the trenched-plots was simulated using a simple model relating decay to surface soil water content. The relation is of the form

$$\frac{dRMR}{dt} = -\kappa RMR \qquad \text{Eqn 1}$$

where t is time in days, RMR is the relative mass of remaining residue (expressed as a fraction of initial mass of residue), and

κ the decay coefficient (d^{-1}), which is a function of soil volumetric water content θ:

$$\begin{cases} \kappa(\theta)=0 \quad if \quad \theta \le \theta_{min} \\ \kappa(\theta)=\dfrac{\theta-\theta_{min}}{\theta_{max}-\theta_{min}} * \kappa_{max} \quad if \quad \theta_{min} \le \theta \le \theta_{max} \\ \kappa(\theta)=\kappa_{max} \quad if \quad \theta \ge \theta_{max} \end{cases} \qquad \text{Eqn 2}$$

The coefficient κ varies between 0, when soil moisture is lower than θ_{min} (a limit under which it is assumed that no significant decomposition occurs), and κ_{max}, an empirical constant which represents the maximum decay rate when soil water content is no longer limiting (i.e. higher than θ_{max}). Between these two limits, the response to soil water content is represented as a linear increase.

On a daily basis, the relative mass of root residues remaining on day i is calculated following:

$$RMR_i = RMR_{i-1} \exp\left[-\kappa(\theta_i)\right] \qquad \text{Eqn 3}$$

This model was calibrated using decomposition data collected by Kazotti (2003), who recorded over 1 yr the evolution of the mass of coarse, medium and fine roots placed in litter bags buried in the soil at a depth of 10 cm, in a nearby *Eucalyptus* plantation. The model (calibrated separately for coarse, medium and fine roots) was applied to the trenched-plot initial root biomass (obtained using allometric relations) and soil water content data recorded in 2005 during the soil respiration measurements, in order to estimate the remaining mass of decomposing roots per m^2 at each date i (MR_i, g m^{-2}). The decomposition CO_2 flux ($F_{d,i}$, µmol m^{-2} s^{-1}) for each measurement date was then estimated according to Epron *et al.* (1999):

$$F_{d,i} = e \cdot c \cdot \tau \cdot (MR_i - MR_{i-1}) \qquad \text{Eqn 4}$$

Microbial efficiency ($1 - e$) was set at 0.2 (Jenkinson, 1990), c the C content of the roots was measured as 0.47 g C g^{-1} DM, and τ (0.9645) is a conversion factor (converting g C m^{-2} d^{-1} to µmol m^{-2} s^{-1}).

The R_h for each measurement date i was then estimated using the measured average CO_2 efflux of trenched plots ($R_{TP,i}$) and the decomposition flux:

$$R_{h,i} = R_{TP,i} - F_{d,i} \qquad \text{Eqn 5}$$

A model relating respiration to soil volumetric water content in the 0 to 6 cm depth zone (see the Results section, Eqn 12) was adjusted to the twice-monthly estimations of R_h and R_s. The daily soil water content data for the month of June 2005 were fed into this model, allowing the estimation of daily R_h, R_s and finally R_{ar} as the difference between the two. The fact that R_h was simulated using soil water content data from normal plots means that it was not necessary to apply a correction for the different soil water content because of reduced water extraction in the trenched-plots. Average monthly R_{ar} was computed as the mean of the daily estimations.

Estimation of the stand level autotrophic component by upscaling direct root respiration measurements

Root respiration measurements Root CO_2 efflux F_c (µmol m^{-2} s^{-1}) was measured on the field on 95 samples of fine roots and 95 samples of coarse roots from April to June 2005, at the ambient temperature and CO_2 concentration of the air at the soil surface. The roots were enclosed in a chamber linked to an infrared gas analyser (IRGA) (EGM, PP systems, Hitchin, UK) in a closed-path system, ensuring that leaks were negligible, and the CO_2 concentration was recorded every 5 s, for a total duration of 255 s.

Fine root samples were composed of several root sections with a diameter smaller than 2 mm, which were extracted from surface portions of soil of 30 cm by 30 cm and 15 cm deep, then rinsed in water, blotted and selected (in order to discard dead or too-short fragments). These samples were placed in a chamber of volume 0.25 l equipped with a ventilator and thermocouple probe, and the respiration measurements were made in the field approx. 5 min after excision.

The diameter of the coarse roots ranged from 2 to 32 mm and the measured portions were located at a lateral distance of 0.3–2 m from the trunk and up to 0.5 m deep. A home-made PVC chamber composed of two half-boxes with semi-circular openings, similar to one used on stems (Damesin *et al.*, 2002), was placed around a root portion of 10 cm in length, which stayed intact and attached to the root system during the measurement. The average diameter was calculated as the arithmetic average of the diameters measured with a vernier caliper on two radii at each end of the segment enclosed in the cuvette. The volume and surface area of the root segment was computed from the diameter of each extremity assuming that the root was cone-shaped. Coarse-root respiration was expressed either on a mass basis (R_m, µmol kg^{-1} s^{-1}) or on a volume basis (R_v, mmol m^{-3} s^{-1}) by dividing F_c, respectively, by the mass or the volume of the sample. The density of fine root tissues was estimated on a subsample, and used to translate rates per unit mass ($R_{fr,m}$) to rates per unit volume ($R_{fr,v}$).

Temperature corrections were applied to our data for two main purposes. First, because of our inability to control the temperature of our measurement chambers in the field, our respiration measurements showed a temperature trend (measurements were made between 08 : 00 h and 15 : 00 h and the temperature of the air at the surface of the soil could vary by as much as 10°C in the course of 1 d). In order to compare the different measurements we therefore normalized all the respiration data to 30°C (mean temperature of the ambient air at which measurements were made). Second, the simulated respiration rates obtained for the upscaling process

needed to be temperature corrected in order to represent true root conditions, i.e. soil temperatures measured during the study period.

The effect of rapid changes in ambient temperature on root respiration was represented by a simple Q_{10} relationship, in which $R(T)$ is the respiration rate at the temperature T, and R_{30} is the rate at the reference temperature of 30°C:

$$R(T) = R_{30} * Q_{10}^{\frac{T-30}{10}} \quad \text{Eqn 6}$$

The Q_{10} parameter represents the sensitivity of the respiration rate to a change in temperature. We did not dispose of the necessary equipment to control the temperature in the measurement chambers, so we used a Q_{10} value of 2.2, which was observed for *Eucalyptus* grown in root boxes (A. Thongo M'Bou, unpublished).

A model relating coarse-root respiration ($R_v(d)$, expressed on a volume basis) to root diameter (d) was fitted to the experimental data:

$$R_v(d) = \frac{2 \cdot R_{vm}}{k^2 \left(\frac{d}{2}\right)^2} \left[\exp\left(-k\frac{d}{2}\right) + k\frac{d}{2} - 1 \right] \quad \text{Eqn 7}$$

This model (see Supplementary Material for details) is based on the hypothesis that the respiration rate of root tissues decreases exponentially (with an exponential coefficient k in m^{-1}) from the external part of the root (where tissues are assumed to contain the highest proportions of living cells and to have the highest respiration rate, R_{vm}) to the central part of the root. We constrained the fit by considering that the respiration rate of roots of diameter 1 mm ($R_v(1)$) was $R_{fr,v}$, the rate (on a volume basis) measured on fine roots. The fit was performed using the Matlab 7.0 nlinfit function which estimates the coefficients of a non linear regression, using least squares estimation.

Upscaling to the stand level Underground biomass estimates were made thanks to allometric relations developed as part of the EucalyptDendro model (Saint-Andre *et al.*, 2002, 2005). Root biomass was also measured in June 2005 thanks to a series of soil cores, made on four plots with nine sampling points per plot (following the same repartition as for soil respiration measurements made during the same period), and four depths per sampling point (0–30 cm, 30–60 cm, 60–90 cm, 90–120 cm). Roots were separated into three diameter classes: < 2, 2–5, and 5–10 mm. These measured biomasses were compared with the output of the EucalyptDendro model. For the 0–5 and 5–10 mm diameter classes, the soil core measurements were in very close agreement with modeled values, which were thus validated as good estimations of the true root biomass. We accordingly decided to work with the modeled root biomasses (given for each tree, depending on its size), which allowed us to calculate root respiration per tree.

Height (h) and diameter at breast height (D) measurements were made on the central trees and immediate neighbors of the three soil respiration blocks (49 trees in total). We used allometric relations between D and h and underground biomass, presented in Saint-Andre *et al.* (2005), to estimate the biomass of roots of three diameter classes (0–5, 5–10 and > 10 mm) for each of the trees in June 2005. To separate the 0–5 mm diameter class into roots < 2 mm and larger ones, we applied the ratio found in the soil cores. Biomass of coarse roots was converted to volume thanks to measurements made on sampled roots: mean fresh volume to dry mass ratio was 2.61 10^{-3} m^3 kg^{-1} for coarse roots (and 2.27 10^{-3} m^3 kg^{-1} for fine roots).

An extensive study of root architecture was undertaken on two similar *Eucalyptus* stands aged 3 yr and 6 yr in 2002. On both stands the entire root systems of three trees, representative of three classes of diameter at breast height, were excavated, and the length and initial diameter of every root of > 10 mm diameter close to the stump were measured. Thanks to this set of data the distribution of root volume according to discrete diameter classes was examined. Making the assumption that roots are conical, and taking into account the individual lengths, each root i was divided into segments of given diameters d (with a diameter increment of 1 mm between steps), of volume $v_i(d)$. The total volumes of the 2–5, 5–10 and > 10 mm classes for the entire root system (V_{2-5}, etc.) were obtained by summation of the corresponding $v_i(d)$, of all the roots, following (e.g. for the root class 2–5 mm):

$$V_{2-5} = \sum_{i=1}^{n} \sum_{d=2}^{5} v_i(d), \quad \text{Eqn 8}$$

(where n is the number of measured roots). The ratio of the volume of a given diameter step and of the volume of the corresponding diameter class, $r(d)$, was used as an estimation of the proportion of total tree root volume associated to each diameter. This proportion was multiplied by the volume deduced from biomass measurements ($V_{tree,2-5}$, $V_{tree,5-10}$, etc.), to obtain an estimate of the true root volume associated with each diameter (e.g. for roots of the 2–5 diameter class):

$$V_{tree}(d) = r(d) * V_{tree,2-5} \quad \text{Eqn 9}$$

This multiplication was necessary because the length and diameter measurements were only made on roots whose initial diameter was > 10 mm, which means that the total volume calculated from these measurements did not take into account the contribution of smaller roots.

Coarse-root respiration rates at 30°C for each diameter $R_{v,30°C}(d)$, were calculated thanks to the fitted model of respiration according to diameter (Eqn 7). Temperature corrected coarse- and fine-root respiration rates were then calculated for each half hour interval in June 2005, applying Eqn 6 to $R_{v,30°C}(d)$ and $R_{fr,m,30°C}$, and averaged in order to

obtain estimations representative of the whole period ($R_v(d)$ and $R_{fr,m}$). Fine root biomass per tree M_{fr} was calculated using the allometric relations presented in Saint-Andre *et al.* (2005).

Total root respiration per tree R_{tree} for each of the six sampled trees was then estimated as:

$$R_{tree} = M_{fr} * R_{fr,m} + \sum_{d=2}^{d\max} R_v(d) * V_{tree}(d) \quad \text{Eqn 10}$$

A simple linear relationship between a tree's root respiration and its D^2h (the product of tree height by the square of its diameter at breast height, in m^3) was fitted to the six points, with two empirical parameters a_1 and a_0:

$$R_{tree} = a_1 * D^2 h + a_0 \quad \text{Eqn 11}$$

This relationship was applied to the trees representative of the three soil respiration blocks, and multiplied by stem density in order to obtain an estimate of the total root respiration per m^2.

Uncertainty and sensitivity analysis

Three different tools were used to examine the uncertainty on our estimations of root respiration: inter-block variance was used to assess the uncertainty due to spatial extrapolation; a Monte-Carlo type analysis was carried out in order to evaluate the precision of the estimations made on each block, taking into account the whole chain of uncertainties involved in our calculation of root respiration; a sensitivity analysis was conducted in order to determine which parameters need to be known with the best precision in order to reduce the uncertainty on the final estimate.

Uncertainty on spatial extrapolation First, interblock variance was calculated and assumed to represent all the subsampling error (Giardina & Ryan, 2002), i.e. error caused by spatial variations of the input data. Following the analysis of Giardina & Ryan (2002), it was assumed that intraplot variance did not contribute to overall variance. The 95% confidence intervals on the estimations of a mean value for the stand were calculated using the interblock variance, and were examined in order to compare the two upscaling methods.

Methodological uncertainties Second, a Monte-Carlo type analysis (Sicard *et al.*, 2006) was carried out on both procedures (upscaling of direct measurements on roots compared with use of soil respiration measurements) in order to estimate the standard deviation on the set of final estimates of root respiration. For the analysis of the direct technique involving upscaling of measurements made on roots, probability density functions were constructed for the different inputs. The $R_{fr,m}$ and the volume per unit of mass were generated randomly from the normal distribution that was fitted to measured values; and all size measurements (D, h, lengths and diameters of all roots of each of the six trees) were assumed to be affected by a random error following a normal distribution of mean 0 and of standard deviation 5% of the measured value. For each of 10 000 simulations, a random set of these inputs was generated. The k parameter of Eqn 7 was then fitted to the experimental data for each random value of $R_{fr,m}$, and volumes of root classes (used in Eqns 8 and 9) and the residual error on these volumes were calculated using the equations given in Saint-Andre *et al.* (2005). Tree coarse-root respiration was calculated for each of the six sampled trees (using Eqn 10) and the six values were used to fit the a_0 and a_1 parameters of Eqn 11, which was then applied to the trees present on the soil respiration plots. Fine-root respiration was computed using the random value of $R_{fr,m}$ and added to coarse-root respiration to yield an estimate of total root respiration.

In the case of the estimation of root respiration using soil respiration measurements and trenched-plots, the same approach was used, applying a random error to the initial root biomass, the parameters of the decomposition function, and to the soil respiration and water content data (using measured standard deviations). For each simulation a set of parameters of the relations between soil water content and R_s and R_h were calculated, and applied to daily soil water content data, to which a random error was assigned, for the obtention of one estimation of R_s, R_h and R_{ar}.

A set of 10 000 estimations was made for each of the three blocks. The standard deviations obtained on each set of 10 000 estimations were taken to represent the uncertainties associated with the methods used to estimate root respiration, taking into account both input data uncertainty and model parameterization.

Sensitivity analysis A simple sensitivity analysis based on the constant fraction approach (Medlyn *et al.*, 2005) was carried out in order to determine which inputs had the most influence on the final output. For each input, a set of 40 estimations was made, with the value of the input varying by 1% steps from a factor of 0.8–1.2 of the value used.

Results

Soil respiration and estimation of its autotrophic and heterotrophic components

Soil respiration R_s was significantly lower in trenched plots than control plots and showed a marked correlation with soil water content. A sigmoidal model of soil respiration as a function of soil volumetric water content (θ),

$$R_s = \frac{a\theta^{\eta}}{b^{\eta} + \theta^{\eta}}, \quad \text{Eqn 12}$$

where a, b and η are empirical parameters, was fitted to the experimental data corresponding to the period from April

Table 1 Biomass, volume and measured respiration rates of *Eucalyptus* roots of four diameter classes

	Biomass (g m^{-2})		Volume (cm^3 m^{-2})		Measured respiration rates (nmol g^{-1} s^{-1})		
Root diameter class (mm)	μ	σ	μ	σ	μ	σ	*n*
0–2	193	22	440	49	10.32	2.54	95
2–5	67	7	174	20	4.24	1.97	12
5–10	59	10	154	25	2.37	1.34	23
> 10	535	171	1395	447	0.80	0.46	60

The biomasses are calculated using the EucalyptDendro model (Saint-Andre *et al.*, 2005), applied to the trees of the soil respiration plots and considering a density of 750 trees ha^{-1}, and are converted to volume using measured root density. The respiration rates are normalized to 30°C using a Q_{10} of 2.2. Means (μ), standard deviations (σ) and numbers of samples (*n*) are presented.

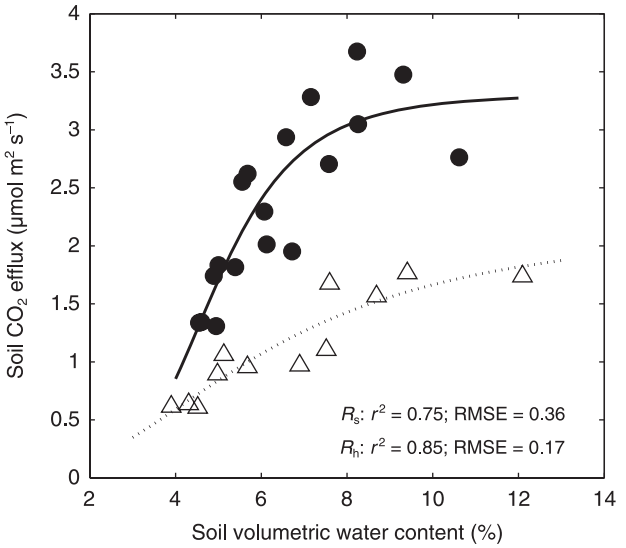

Fig. 1 Soil respiration R_s (circles) and estimated heterotrophic respiration R_h (triangles; after correction for decomposition) versus soil volumetric water content at 0–6 cm in 2005 (pooled data of the three blocks). The lines show the fit of Eqn 12 to the experimental data. The coefficient of determination between observed and simulated values (r^2) and root mean square error (RMSE, μmol m^{-2} s^{-1}) of each fit are presented.

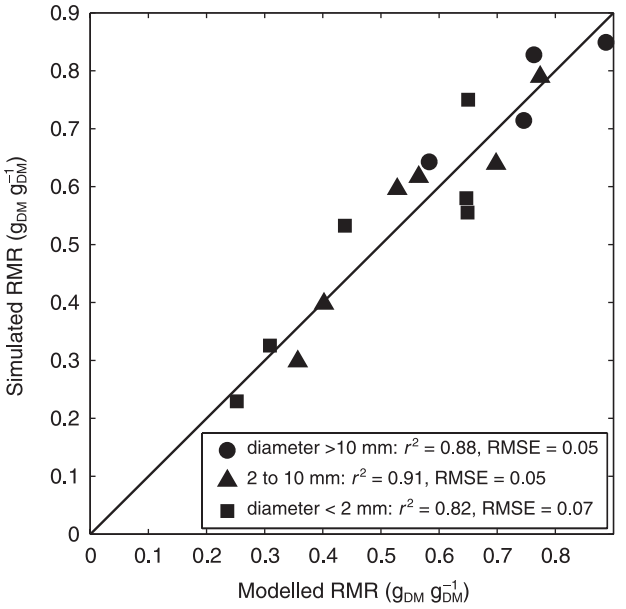

Fig. 2 Measured versus simulated remaining masses of root residues (RMR) using the decomposition model, for three diameter classes (circles, > 10 mm; triangles, 2–10 mm; squares, < 2 mm). The diagonal line represents the 1 : 1 line.

2005 to September 2005, and explained 75% of the temporal variability of soil CO_2 efflux measured on control plots (Fig. 1). Adding soil temperature as a variable in the model did not improve the fit, which corroborates the findings of a previous study of soil respiration in these plantations (Epron *et al.*, 2004). In this model, a theoretical asymptote is reached (at a respiration level primarily controlled by the *a* parameter), which would mean that above a certain soil volumetric water content (controlled by the *b* parameter), soil respiration no longer depends on humidity. The η parameter controls the shape of the relationship.

The calibration of the decomposition model gave a good fit between simulated and measured data (r^2 of 0.82 to 0.91 and RMSE (root mean square error) of 0.05–0.07, depending on the root diameter class, Fig. 2). R_h, computed as the difference between the measured soil CO_2 efflux from trenched plots and the simulated decomposition flux, was also well correlated to soil water content. Equation 12 was fitted to R_h and trenched plot soil water content data and explained 85% of the temporal variations between April and September 2005 (Fig. 1).

Daily values of control plot soil volumetric water content were fed into fitted Eqn 12 and yielded daily estimates of R_s and R_h, and by subtraction R_{ar}. Using the Monte-Carlo approach, means and standard deviations of simulations of the average monthly values were determined for each block and are presented in Table 2. The average estimate of R_{ar} was 1.05 μmol m^{-2} s^{-1}, which corresponds to a root contribution of 48%, but the high standard deviations obtained with the Monte-Carlo simulations (0.28–0.30 μmol m^{-2} s^{-1}) reflect the high uncertainty on this value. The 95% confidence

Table 2 Estimations of *Eucalyptus* root respiration in June 2005 per unit soil surface, on three blocks made up of one trenched-plot and one control plot

Result of 10 000 simulations (μmol CO_2 m^{-2} s^{-1})	Upscaling root respiration measurements			Using soil respiration measurements			
Block	Coarse root respiration	Fine root respiration	Total root respiration	Soil respiration	Heterotrophic component	Total root respiration	Difference
1	0.43 (0.15)	1.04 (0.25)	1.47 (0.21)	2.13 (0.25)	1.08 (0.17)	1.06 (0.28)	0.41
2	0.48 (0.18)	1.11 (0.27)	1.59 (0.22)	2.16 (0.20)	1.23 (0.22)	0.95 (0.27)	0.64
3	0.46 (0.17)	1.08 (0.26)	1.54 (0.21)	2.23 (0.20)	1.09 (0.24)	1.16 (0.30)	0.38
Mean	0.46 (0.03*)	1.08 (0.03*)	1.53 (0.06*)	2.18 (0.05*)	1.13 (0.08*)	1.05 (0.10*)	0.48 (0.14*)

Values are means (SD) of the 10000 simulated values. Both the results of the approach based on soil respiration measurements and those of the upscaling of root respiration measurements are presented, as well as the difference between the two.
*Means (SD) of the three block estimations.

interval on the mean value, calculated using inter block variance, is (0.85; 1.25) μmol m^{-2} s^{-1}.

The mean simulated decomposition flux during the month of June 2005 was 0.18 μmol m^{-2} s^{-1}, which corresponded to the disappearance of 66 of the 1393 g m^{-2} of initial root mass between April 14 2005 and June 30 2005.

The sensitivity analysis showed that the inputs of the decomposition model (parameters and initial root biomass) only had a small influence on the estimation of R_h (a 10% change in input parameters only changed calculated R_h by 3% and therefore R_{ar} by < 3%). Conversely, the parameters of the fit of R_s to humidity data had a critical effect on the final result: a 10% change in the *a* and *b* parameters respectively caused a 19% and 30% change in the stand root respiration estimate.

Estimation of the autotrophic component using root respiration measurements

Measured respiration rates of roots of four diameter classes are presented in Table 1. Mean fine-root respiration at 30°C was 10.3 μmol kg^{-1} s^{-1}, with a standard deviation of 2.54 μmol kg^{-1} s^{-1}. Coarse-root respiration rates expressed on a mass or volume basis showed a strong relation with root diameter (Fig. 3) and a good fit of Eqn 7, which explained 77% of the observed variability. When the fit was constrained assuming that roots of diameter 1 mm respired at the mean rate measured on fine roots, the estimated value of the *k* parameter was 6672 m^{-1}, and the corresponding value of R_{vm} was 10.6 mmol m^{-3} s^{-1}.

To scale these results up to the stand level, we used an approach that took into account the contribution of the different root diameters to the total volume. The structure of the stand was also taken into account: the calculation of respiration was made for six individual trees, and scaled up to the stand level by using a fitted relationship between root respiration and size of the tree (diameter at breast height (*D*) and height (*h*)).

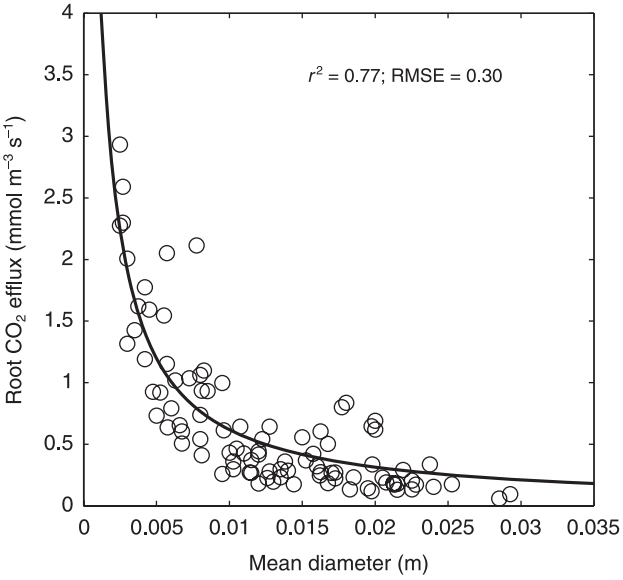

Fig. 3 Respiration (per unit of root volume) vs diameter as measured (circles) and modeled with Eqn 7 (line) on a 3-yr-old stand planted with the *Eucalyptus* UG clone. The coefficient of determination between observed and simulated values (r^2) and root mean square error (RMSE) of the regression are presented.

The estimated respiration rates per tree showed a significant exponential trend according to *D*. The fit became linear if respiration was plotted against D^2h (Fig. 4). We applied this linear relation to the trees surrounding the soil respiration plots, whose height and diameter at breast height were measured in June 2005.

After temperature correction using half-hourly soil temperature measurements, the mean over the three blocks of total root respiration was estimated at 1.53 μmol m^{-2} s^{-1}, with a standard deviation of only 0.06 μmol m^{-2} s^{-1} between blocks (Table 2), resulting in a confidence interval on the mean of (1.41; 1.65) μmol m^{-2} s^{-1}. Three quarters of the total respiration was produced by fine roots. If this estimate is compared

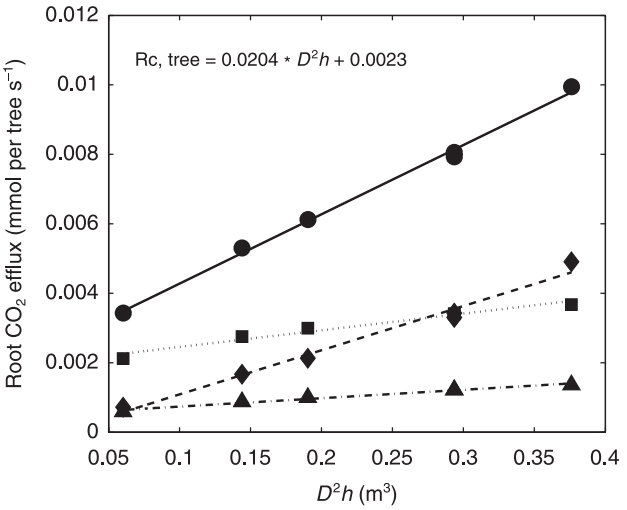

Fig. 4 Estimated coarse-root respiration per tree, compared with D^2h (product of the tree height and the square of tree diameter at breast height), in total (circles), and for the 2–5 mm (squares), 5–10 mm (triangles) and > 10 mm (diamonds) root diameter classes. The equation of the linear regression between total coarse root respiration and D^2h is presented.

with the estimate of total soil respiration over the same period, it corresponds to an autotrophic contribution of 70%.

The Monte-Carlo simulations made on each block resulted in high standard deviations (0.21–0.22 μmol m^{-2} s^{-1}), which reflect the poor precision of the estimates owing to the numerous uncertainties involved in the estimation process, which are not only linked to interblock variation.

The sensitivity analysis examined the effect of changes by steps of 1% of each input on the total coarse-root respiration. The parameters describing the trees (i.e. height and particularly diameter) have a big influence on the result because they are used in the allometric relations to determine the volume of root material. A 10% difference in mean diameter at breast height leads to a > 8% difference in the estimate of stand total root respiration (the difference is larger for the coarse roots); nevertheless such a measurement error is highly implausible. The parameters of the linear relation between coarse-root respiration and tree size have a much smaller influence: although their combined effect on coarse-root respiration is directly proportional, they have no effect on the estimation of fine-root respiration which is by far the larger part of total respiration. In fact, one of the most critical parameters is $R_{fr,m}$ (i.e. the fine-root respiration rate). This input is associated with a high standard error and has a direct effect on both coarse- and fine-root respiration estimations. With all else equal, a 10% error in the estimation of $R_{fr,m}$ leads to an equally large error in the final estimate both of fine- and coarse-root respiration. The temperature correction is the other critical parameter, as it has a direct, exponential influence on the final estimation. This highlights the need for extensive temperature measurements for any attempt at temporal extrapolation. The importance of the relationship used for temperature correction, of the correct estimation of the Q_{10} value and of its possible variations, must also be stressed.

Discussion

The detailed description of root architecture available for the Congolese *Eucalyptus* plantations enabled us to use the relationship between root diameter and respiration to produce an estimation of root respiration at the stand level, taking into account the structure of the root system. This upscaling method provides an independent estimate of root respiration at the stand level that can be compared with a different estimation technique, which implies the measurement of soil respiration on root exclusion and control plots.

Using the indirect trenched-plot technique we obtain an estimate of 1.05 μmol m^{-2} s^{-1} for total rhizospheric respiration during the month of June 2005, i.e. *c.* 70% of the estimation we have made by up scaling direct measurements made on roots (1.53 μmol m^{-2} s^{-1}). The confidence intervals on these two mean values, taking into account spatial (interblock and intrablock) variability, do not overlap.

Total soil respiration was estimated at 2.18 μmol m^{-2} s^{-1} in June 2005, giving a root contribution of 48% using the root exclusion technique and 70% using the direct measurement and up scaling technique. These estimates are in the range recorded in literature (Hanson *et al.*, 2000) and they are in agreement with the 59% estimate obtained by comparing soil respiration in an intact area and a clear cut area of an older nearby *Eucalyptus* stand (Epron *et al.*, 2006). The fact that a significantly higher value is obtained using the direct measurement technique contradicts the observation of a review on the separation between R_h and R_{ar} (Subke *et al.*, 2006), which shows that root excision and measurement techniques tend to give lower estimates of R_{ar} than the techniques involving the subtraction of R_h from R_s. Our trend is a little surprising, as one would expect R_{ar} derived from indirect techniques to comprise a larger rhizospheric component (respiration of microorganisms that depend on substances produced by living roots) and therefore to be a little higher than R_{ar} estimated from direct measurements on extracted (and generally washed) roots. Nevertheless, the numerous sources of uncertainty associated with both methods mean that the results should be interpreted with caution.

Our Monte-Carlo analysis, which aims to assess the methodological uncertainties contained in each estimation, provided distributions of estimations of root respiration with high standard deviations, and what is more it suffered from an inherent bias: namely, that the distribution of fine-root respiration rates was assumed to be represented by measured rates. A possible large systematic error on these measurements was therefore not taken into account in the Monte-Carlo analysis. And yet, as our sensitivity analysis showed, the upscaling technique applied for coarse roots was not the

largest source of possible error in the calculation of total stand respiration: the most critical input for these *Eucalyptus* stands was the respiration rate of fine roots and their temperature sensitivity. This is simply due to the fact that the fine root contribution represented 75% of the total root respiration flux. The causes for uncertainty on these measurements are numerous, including CO_2 effects (Burton *et al.*, 1997; Clinton & Vose, 1999), instrumentation (leaks, Burton & Pregitzer, 2002), and disturbance and wounding effects. Excision of fine roots is one of the most probable causes of a possible overestimation of root respiration in our study (Rakonczay *et al.*, 1997). Our accounting for temperature sensitivity must also be treated with caution, as the Q_{10} value has a big influence on the final estimation. Temperature sensitivity of root respiration is still not very well understood; recent work has attempted to explicit the mechanisms underlying the short-term effect of temperature (Atkin *et al.*, 2000) and to prove the existence of longer-term acclimatization (Loveys *et al.*, 2003). We only attempted to correct our results empirically for the immediate effect of surrounding temperature on the roots, but it is probable that temperature (of soil or air) exerts other more subtle effects that vary with the season and plant activity.

Based on our observations of root respiration, we used root diameter as an upscaling variable, thus neglecting other possible factors such as N content or root depth. In our study, although a correlation was observed between N content and respiration rates, adding N content as a variable did not improve the relationship between diameter and respiration (results not shown). This may be due to the low N content gradient in our samples.

In a study on sugar maple roots, Pregitzer *et al.* (1998) concluded that there were significant negative relationships between the respiration of a root and its diameter, but also its depth (independently of temperature gradients that exist in the soil profile). We did not take this depth effect into account, as all of our fine-root respiration measurements were made on samples collected in the first 15cm of soil, and none of our coarse roots was sampled any deeper than 50 cm. In Pregitzer's study, the difference was only important for roots smaller than 0.5 mm in diameter, whose respiration rates differed by > 50% between the 0–10 cm and 20–30 cm soil layers. If we consider that one-quarter of our fine roots belong to this diameter class and therefore in the deeper layers respire at half the rate that we measured, and that the top 15 cm of soil only contain one-quarter of their biomass, our estimation of total root respiration drops by *c.* 6%. One could also expect that temperature gradients in the soil profile would have to be taken into account for the temporal extrapolation, when temperature corrections are applied. In our case the temperature gradient was very limited between surface soil and deeper soil in June 2005, so such a manipulation was not necessary, but during seasons with more contrasted conditions the temperature profile of the soil would have to be accounted for.

The results of our simulations indicate that the uncertainties on the result of the upscaling technique, although high, are lower than those associated with the root exclusion approach. A large part of the uncertainties in the indirect approach resulted from the strong impact of the parameters of the function relating respiration to soil humidity, which was used for the temporal interpolation of soil respiration measurements. The parameters of this function were fitted on the basis of measurements made between 08 : 00 h and 15 : 00 h, a period which might not correctly represent the whole day, and at a twice-monthly time-step which might not be sufficient to capture the response of the system to rapid variations in soil humidity, or to other factors such as, for example, tree photosynthetic activity. It might be for these reasons that the function used for interpolation only explained 75% of the temporal variability observed. More extensive studies on diurnal and day-to-day variations of fluxes, using continuous soil respiration measurements, should be carried out to gain more insight into these aspects, which could have a significant impact on long-term estimations of CO_2 fluxes.

Other possible systematic errors should be taken into account. When using the root exclusion approach, if the heterotrophic component is overestimated, the estimation of root respiration will be too low. Such an overestimation is quite possible, for example owing to insufficient correction of the contribution of decaying roots to the CO_2 efflux of trenched plots (Epron *et al.*, 1999). We considered that the contribution of the stump was negligible, but if we consider that it decomposes in the same way as coarse roots, the estimation of R_{ar} is slightly higher. Furthermore it is likely that surface soil moisture was much lower than in deeper horizons during the period considered, because trenching stopped transpiration without stopping evaporation. As the decomposition flux was calculated using surface (0–6 cm) soil water content measurements, it could have been underestimated.

In root-exclusion experiments, inputs of C through litterfall and fine-root turnover are expected to decrease (Epron *et al.*, 2006). In our case, the litterfall effect must be limited because the surrounding trees continue to shed litter, whereas the effect of the cessation of fine root turnover might be more significant.

It is also possible that our soil respiration measurements systematically underestimated real fluxes. Soil respiration measurements were made by placing the soil chamber on PVC collars that cut through the root mat when they were installed. This could mean that a part of the autotrophic component is not measured, as shown in a study on a larch forest (Wang *et al.*, 2005). However, in this study the collars were installed more than a month earlier, and we verified that there was no significant difference between measurements made on 1-month old-collars and 1-yr-old collars, and that after 1 yr the soil within collars was recolonized with roots. There is also an ongoing controversy about the accuracy of different soil respiration measuring devices because of importance

of the design of the chamber and the delicate physical phenomena inherent to the soil–litter–atmosphere boundary layer (Le Dantec *et al.*, 1999; Janssens *et al.*, 2000, 2001; Longdoz *et al.*, 2000; Rayment & Jarvis, 2000; Yim *et al.*, 2002; Ngao *et al.*, 2006).

In our study the precision offered by the technique involving upscaled root respiration measurements (represented by the standard deviation on the Monte-Carlo simulations) was a little better than that produced by the trenched-plot technique, thus justifying the use of direct measurements for upscaling of root respiration. More progress is nevertheless needed in the conception of a less intrusive measurement technique of fine-root respiration, and in the description of the effect of environmental, morphological and phenological factors on respiration rates. Relating respiration to root characteristics opens the way for linkage of a respiration model with a structural model of root development, which could allow the dynamic simulation of root CO_2 efflux along the lifetime of the plant.

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Supplementary material

The following supplementary material is available for this article online:

Text S1 Model proposed in Eqn 7 for the respiration of a root segment versus its diameter.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02300.x
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