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Tree-girdling to separate root and heterotrophic respiration in two *Eucalyptus* stands in Brazil

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Abstract The release of carbon as CO₂ from below-ground processes accounts for about 70% of total ecosystem respiration. Insights about factors controlling soil CO₂ efflux are constrained by the challenge of apportioning sources of CO₂ between autotrophic tree roots (and mycorrhizal fungi) and heterotrophic microorganisms. In some temperate conifer forests, the reduction in soil CO₂ efflux after girdling (phloem removal) has been used to separate these sources. Girdling stops the flow of carbohydrates to the below-ground portion of the ecosystem, which should slow respiration by roots and mycorrhizae while heterotrophic respiration should remain constant or be enhanced by the decomposition of newly dead roots. Therefore, the reduction in CO₂ efflux after girdling should be a conservative estimate of the belowground flux of C from trees. We tested this approach in two tropical *Eucalyptus* plantations. Tree canopies remained intact for more than 3 months after girdling, showing no reduction in light interception. The reduction in soil CO₂ efflux

averaged 16–24% for the 3-month period after girdling. The reduction in CO₂ efflux was similar for plots with one half of the trees girdled and those with all of the trees girdled. Girdling did not reduce live fine root biomass for at least 5 months after treatment, indicating that large reserves of carbohydrates in the root systems of *Eucalyptus* trees maintained the roots and root respiration. Our results suggest that the girdling approach is unlikely to provide useful insights into the contribution of tree roots and heterotrophs to soil CO₂ efflux in this type of forest ecosystem.

Keywords Belowground production · Net ecosystem production · Tropical forest plantation

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Introduction

The efflux of carbon as CO₂ from the soil surface ('soil respiration') is the major pathway of carbon loss in forest ecosystems (Raich and Potter 1995; Goulden et al. 1996; Law et al. 1999; Janssens et al. 2001). Both autotrophs (roots and mycorrhizal fungi receiving plant carbon) and heterotrophs (microbes) contribute to the efflux of CO₂ from the soil. Separating the autotrophic and heterotrophic components may be useful because heterotrophic processes control soil carbon storage and nutrient dynamics, while autotrophic respiration reflects plant activity and the supply of organic compounds to roots from the canopy. Estimates of the autotrophic component of soil CO₂ efflux range from 10 to 90%, with a mean for forests of 46% (Hanson et al. 2000). The autotrophic component of soil CO₂ efflux may be greater for high-productivity sites (>60%) than for low-productivity sites (<20%; Bond-Lamberty et al. 2004).

Knowledge of the fraction of soil CO₂ efflux from autotrophs and heterotrophs could also be used to estimate belowground detritus production, an important component for modeling, from estimates of total belowground carbon allocation (TBCA, includes coarse and fine root production and respiration, root exudates,

and plant carbon used by mycorrhizae). From 25 to 60% of gross primary production is allocated belowground (Law et al. 1999; Giardina and Ryan 2002; Stape 2002; Ryan et al. 2004; Litton et al. 2006), and estimates of root production are difficult and expensive and exclude important belowground inputs, such as mycorrhizal turnover and root exudates. The TBCA technique estimates the flux of plant carbon belowground with a carbon balance approach, where belowground production equals the rate of CO₂ efflux from the soil surface minus the C input from aboveground litter, plus or minus any change in storage in the pools of soil and root C (Raich and Nadelhoffer 1989; Giardina and Ryan 2002). TBCA minus autotrophic flux (autotrophic contribution to soil CO₂ efflux) would estimate detritus production, assuming that the storage pools of C were in steady state. Estimates of the fraction of soil CO₂ efflux from autotrophs and heterotrophs can also be used to validate models of belowground processes.

Högberg et al. (2001) used a tree-girdling technique in a Scots pine stand to estimate heterotrophic and autotrophic components of soil CO₂ efflux. Girdling removes the bark and phloem down to the youngest xylem and stops the flow of carbohydrates belowground while maintaining soil temperature and moisture. Högberg et al. (2001) found that soil CO₂ efflux on the girdled plots dropped by one third within 5 days (and by more than one half within 1–2 months), indicating that current tree allocation belowground for root and mycorrhizal respiration accounted for about one half of the current soil CO₂ efflux. Longer monitoring of the girdled plots suggested that root and mycorrhizal respiration accounted for 65% of the soil CO₂ efflux, because girdling had initially enhanced heterotrophic respiration (Bhupinderpal-Singh et al. 2003). Another girdling study in Norway spruce (*Picea abies*) showed a similar 50% reduction in soil CO₂ efflux after girdling (Subke et al. 2004). However, girdling in a mixed deciduous forest did not reduce soil CO₂ efflux (Edwards and Ross-Todd 1979).

This girdling approach provides a minimum estimate of the CO₂ release from roots, mycorrhizae, and the microbes that decompose recent exudates from roots (Högberg et al. 2001) for two reasons: heterotrophic release of CO₂ may increase as microbes decompose freshly killed roots and mycorrhizae, and surviving roots might draw down starch reserves and sustain autotrophic CO₂ release for some period after girdling. Both factors underestimate the autotrophic components shortly after girdling. Over a longer period, the lack of new carbohydrates from the canopies could lower root and mycorrhizal production and detrital input, which would reduce substrate supply for microbes and underestimate the heterotrophic fraction of soil CO₂ efflux relative to intact stands.

The objectives of the investigation reported here were to test this girdling approach its efficiency in separating the autotrophic and heterotrophic components of soil CO₂ efflux in rapidly growing tropical *Eucalyptus*

plantations and to determine if the girdling response was proportional to the fraction of trees girdled within a plot. We hypothesized that girdling trees would provide useful estimates of the autotrophic contribution to soil CO₂ efflux and tested the four following predictions based on this hypothesis in two experiments.

1. *Eucalyptus* trees will remain healthy for at least 3 months after girdling, thereby minimizing confounding effects from girdling that might alter soil microenvironmental conditions and heterotrophic CO₂ production.
2. The reduction in soil CO₂ efflux will be rapid, demonstrating a strong link between current canopy photosynthesis and belowground production.
3. Live root biomass will either not change or will rapidly approach zero, which would support the use of this method as a means to separate autotrophic and heterotrophic contributions to soil CO₂ efflux.
4. The reduction in soil CO₂ efflux will be proportional to the fraction of trees girdled within plots.

Support for predictions 1 and 3 would indicate that the root contribution to soil CO₂ efflux can be estimated with this method. If all four predictions were to be supported by experimental evidence, the girdling approach could be useful for identifying the contribution of a cohort of trees within a stand (such as removing all dominant or all suppressed trees), providing insights on within-stand belowground production to complement aboveground patterns (Binkley et al. 2002). If girdling one half of the trees in a plot provided 50% of the reduction observed from girdling all of the trees, then surviving trees would not have altered their belowground production in response to the girdling of their neighbors. If the response were less than one half of the decline in CO₂ resulting from girdling all trees, then the contribution of the surviving trees could not be separated from the contribution of girdled trees, and this partial-plot girdling would not be useful for estimating the contribution of cohorts of trees to total plot CO₂ efflux.

Site description and methods

Experiment 1 was carried out on a 5-year-old plantation in the northeastern coastal region of Bahia State, Brazil, about 20 km SW of Entre-Rios (11°58'S, 38°07'W). The site is situated 250 m.a.s.l with a mean annual temperature of 25.3°C and an average rainfall of 1040 mm year⁻¹. We used the control plots (not irrigated, not-repeatedly fertilized) of Stape (2002; site also described in Stape et al. 2004), plus an additional set of plots in each of the four blocks for girdling. This second-rotation plantation (following pasture) was established in June 1996 with a clone of *E. grandis* × *urophylla* planted at 3.0×3.0-m spacing. In September of 2001, the trees averaged 16.5 cm in diameter at 1.3 m (diameter at breast height, dbh), 26 m in height, 23 m² ha⁻¹ in

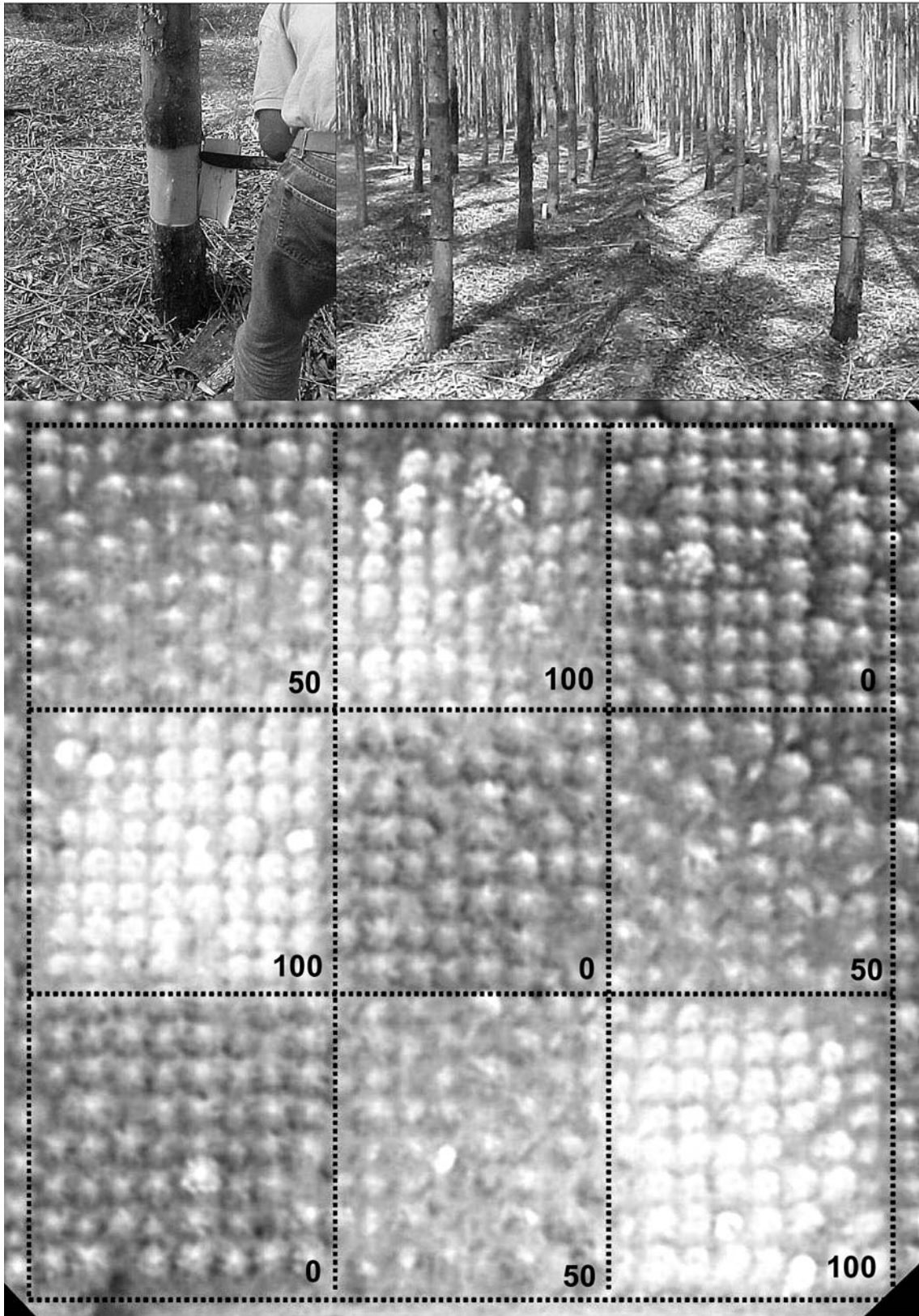


Fig. 1 *Top left* Photo of tree-girdling treatment plot, *top right* Experiment 1 immediately after girdling; *grid photograph* aerial view of Experiment 2 11 months after girdling showing light-

colored canopies without leaves: 0 control, 50 one half of trees girdled, 100 all trees girdled

basal area, and $260 \text{ m}^3 \text{ ha}^{-1}$ in wood volume (approx. 130 Mg ha^{-1}). Understory vegetation was minimal as a result of herbicide applications; any understory contribution to soil CO_2 efflux would be negligible. Four control and four girdled plots ($30 \times 30 \text{ m}$) were calibrated for soil CO_2 efflux for 8 months prior to girdling, with 15 samples taken at permanent points within the central $20 \times 20\text{-m}$ portion of each stand. Based on extensive experimental experience with *Eucalyptus* plantations, we were confident that a buffer of 20 m between measurement plots was more than sufficient to prevent any effect of neighboring plots and treatments. On September 15, 2001, all of the trees were girdled by removing a 0.5-m length of bark from the stem at a height between 0.5 and 1.0 m above the ground (Fig. 1). These *Eucalyptus* species do not root graft, based on our experience with excavating dozens of trees in other studies. Soil CO_2 efflux was monitored on a weekly basis (approximately) for the next 2 months, and then at longer intervals. The condition of the trees was monitored by measuring light interception in all plots: once before girdling and four times after girdling. Light interception was measured with a Ceptometer-AccuPAR Model 80 (Decagon Devices, Pullman, Wash.), with 30 measurement points in each plot and each measurement point consisting of the average of four samples (in cardinal directions).

Soil CO_2 efflux was measured with a PPSystems CIRAS 1 gas analyzer and soil respiration chamber (7800 mm^2 ; PPSystems, Haverhill, Mass.; similar to Giardina and Ryan 2002). The coefficient of variation within plot and sampling day averaged about 40%, with a standard error of the estimate for each plot of about 10–15%. The PPSystems chamber may provide higher fluxes than the LICOR 6400-9 apparatus (LI-COR, Lincoln, Neb.) (Janssens et al. 2000), and Stape (2002) found that the PPSystems chamber gave 26% greater fluxes in a comparison for Experiment 1. We did not adjust the fluxes for this study, because our objectives were to compare differences among treatments.

Fine roots were collected once before girdling and four times after girdling (up to 5 months after treatment). At each collection time, 18 cores ($7.5 \times 45 \text{ cm}$) were taken systematically in each plot, composited within plots, and sorted to remove live roots (identifiable through color and texture) $< 3 \text{ mm}$ in diameter. Roots were dried and weighed; chemical analysis of eight samples from the control stand showed average (standard error) concentrations of 6.3% ash (0.62), 15.4% extractives (0.3), 44.8% lignin (0.8), and 38.8% holo-cellulose (0.9). The respiration of live fine root was measured three times after girdling in the control and girdled plots using sub-samples from the core samples. For each plot, we measured respiration on five sub-samples per plot, with each sub-sample consisting of 14–20 g (wet weight) of roots $< 3 \text{ mm}$. We measured respiration with the PPSystems CIRAS 1 gas analyzer and soil respiration chamber (sealed to prevent mixing with outside air). The CIRAS 1 gas analyzer estimates respiration rate by measuring the rate of increase in CO_2

concentration per unit time for a known volume of air. Root respiration was estimated as (micromoles per square meter per second) $\times$ chamber area (square meters)/sample dry weight.

Experiment 2 was carried out on a 6.5-year-old plantation of *E. grandis* $\times$ *urophylla* clones planted at $3.0 \times 3.0\text{-m}$ spacing. In January 2004, the 1100 trees ha^{-1} averaged 17.4 cm dbh, 26 m in height, $27 \text{ m}^2 \text{ ha}^{-1}$ basal area, and $314 \text{ m}^3 \text{ ha}^{-1}$ wood volume (approx. 157 Mg ha^{-1}). Plot size and methods were the same as in Experiment 1, with eight measurement periods (at 18 permanent points within each plot) prior to girdling on March 28, 2004, and eight measurement periods after girdling in the next 3 months. The completely randomized block design had three replicate plots of three treatments: control (ungirdled), one half of the trees girdled, and all of the trees girdled. The half-girdling treatment entailed girdling every other tree in each row, with adjacent rows offset by one tree to provide uniform spacing between girdled and non-girdled trees.

We analyzed both experiments with an analysis of covariance, testing whether girdling all trees reduced soil CO_2 efflux (Experiments 1 and 2), and whether girdling one half of the trees would provide one half of the effect of girdling all trees (Experiment 2). Analysis of covariance is a more sensitive test than a simple analysis of variance because it explicitly accounts for any pre-existing differences between control and treatment plots and because it accounts for any differences in environment between the pre- and post-girdling periods. The dependent variable was soil respiration for the treated plots, with the control plot in each block for each measurement period as the covariate. We tested for differences in slopes and adjusted means in Experiment 1 between the pre-girdling period (8 months) and two post-girdling periods: 3 months after girdling and 4–9 months after girdling. The analysis was the same for

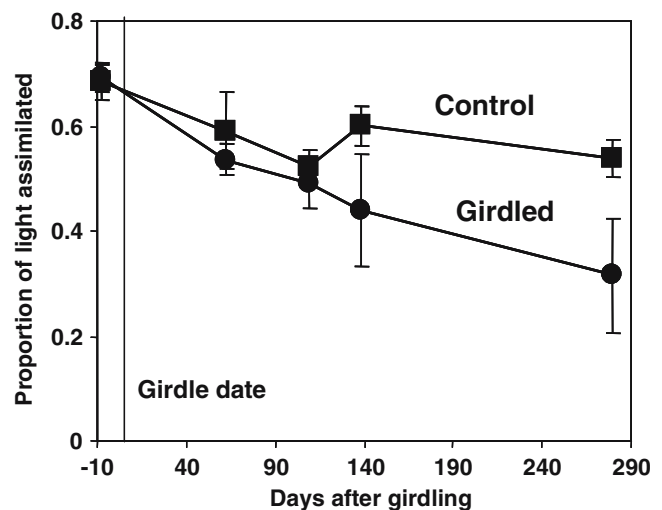


Fig. 2 Light interception in Experiment 1 remained similar between control and girdled plots for more than 100 days after girdling. Bars are standard errors of the means from four plots/treatment

Experiment 2, but with only one post-girdling period (first 3 months). Because measurements were taken on the same plots through time, we accounted for the effect of autocorrelation in time on the analysis using an autoregressive model. We used SAS Proc Mixed (SAS, Cary, N.C.) for the analysis.

Results and discussion

The first prediction was supported based on the high retention of leaves and high light interception in Experiment 1. Light interception did not differ substantially between plots until after 3 months (Fig. 2), so the microenvironment in the soil should not have altered heterotrophic activity. We did not measure light interception after girdling in Experiment 2; however, the canopies showed no discernible reduction in light interception even 6 months after girdling, although the trees

were almost completely leafless after 11 months (Fig. 1). The second prediction was supported in both experiments, as soil CO_2 efflux from the girdled plots was reduced in the 2 weeks between girdling and the first sampling (Fig. 3). Based on earlier results from related studies (Stape 2002), the seasonal and within-month variations in soil CO_2 efflux relate well to vapor pressure deficit (and current rates of photosynthesis). The reduction in CO_2 efflux after girdling was much lower than expected from studies in boreal trees (Högberg et al. 2001) and from a cross-site relationship with productivity (Bond-Lamberty et al. 2004).

In Experiment 1, soil CO_2 efflux averaged $3.1 \mu\text{mol C m}^{-2} \text{s}^{-1}$ for the control plots, and girdling all trees reduced soil CO_2 efflux only by 16% (95% confidence interval from 6.5 to 20%) for 3 months. From months 4 to 9 post-girdling, the girdled plots averaged 6.6% lower soil CO_2 efflux than the control plots, but the difference was not significant ($p = 0.53$). In Experiment 2,

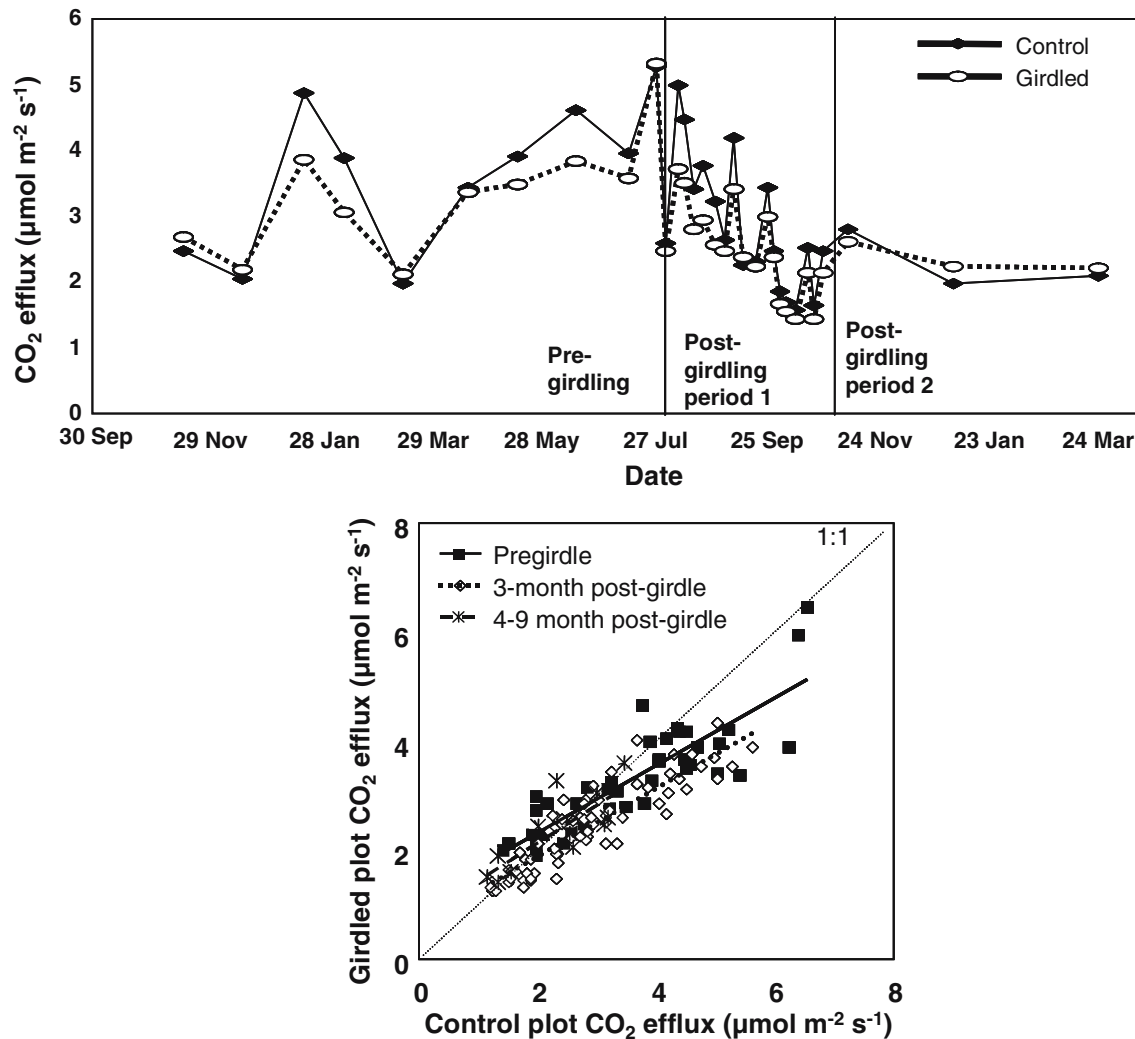


Fig. 3 Soil CO_2 efflux (*upper*) dropped by 15.8% in the first 3 months in response to girdling ($p = 0.01$), but the 6.6% reduction that occurred from 4 to 9 months post-girdling was not significant ($p > 0.5$). The *slopes* of the relationships between control and

girdled plots (*lower*) did not change among periods (each point is an observation comparing the control and girdled plot at one time within one block)

soil CO₂ efflux averaged $1.9 \mu\text{mol C m}^{-2} \text{s}^{-1}$ from the control plots, and girdling all trees lowered soil CO₂ efflux by 24% for 3 months (Fig. 4). In the first experiment, girdling did not affect the slope or Y-intercept in the relationship within plots between the pre- and post-girdling periods. Both the slope and Y-intercept were changed by girdling all trees in Experiment 2. Overall, girdling significantly reduced soil CO₂ efflux ($p=0.04$) across both experiments, but the reductions were less than one half of the anticipated reduction.

The third prediction was refuted, as live fine root biomass sampled in Experiment 1 remained high for at least 3 months after girdling (Fig. 5). Fine root respiration rate also did not decline with girdling (Fig. 6, $p=0.42$) and averaged $2.4 \text{ nmol g}^{-1} \text{s}^{-1}$ for the two sampling periods at 35 and 106 days after girdling.

The fourth prediction was also refuted, as the reduction from girdling one half of the trees (20%) did not

differ from the response of girdling all of the trees (24%; $p=0.22$). Girdling one-half of the trees did not alter the slope between pre- and post-girdling periods, but the Y-intercept did change. The inconsistency in the response relationships between the 50 and 100% girdling treatments resulted in significant interactions between soil CO₂ efflux and sampling period, and CO₂ efflux and treatment. These interactions resulted from one plot in the 100% treatment showing a very high CO₂ efflux before girdling and a large reduction following girdling. The CO₂ efflux from this plot before girdling averaged $0.68 \mu\text{mol C m}^{-2} \text{s}^{-1}$ more than that of the next highest plot, whereas the full range of averages for the other eight plots spanned only $0.57 \mu\text{mol C m}^{-2} \text{s}^{-1}$. If this outlier plot (both pre- and post-girdling) is omitted, the difference in slopes between the pre- and post-girdling periods is removed, and the estimated effect of girdling all trees is reduced from about 24 to 14%, which is close to the 16%

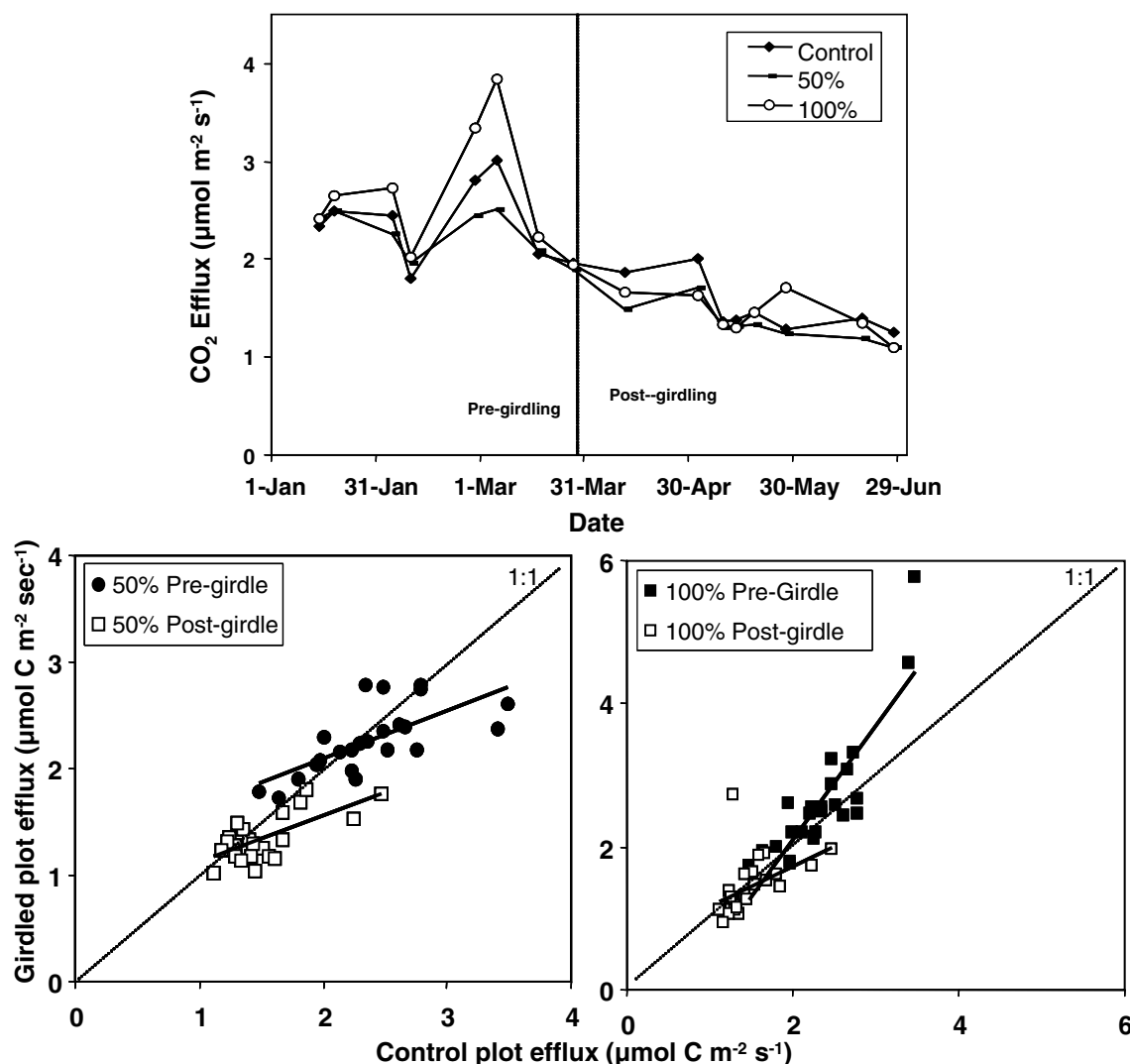


Fig. 4 Girdling of all trees in Experiment 2 reduced soil CO₂ efflux (left) by 24%, and girdling one half of the trees reduced it by 20% ($p=0.02$ for girdling effect; for the difference between 50 and 100% girdling, $p=0.22$). The slopes of the relationships between the

control and 50% girdled plots did not differ between periods (middle), but they differed significantly between periods for the 100% girdled treatments (right)

reduction observed in Experiment 1. We suspect that the 14% reduction in soil CO₂ efflux is probably a better estimate of the mean effect of girdling. However, we chose to retain the outlier plot because removing it did not change the overall statistical conclusions that girdling reduced CO₂ efflux and that the effect of girdling one half or all of the trees did not differ.

The success of the girdling approach to identifying the contribution of current root activity to soil CO₂ efflux depends on at least two factors: (1) the release of CO₂ from roots and associated mycorrhizae must drop quickly, and (2) the girdling treatment must stop root activity without stimulating heterotrophic activity (as might happen if freshly dead roots decomposed rapidly). These conditions appear to have been met in the study by Högberg et al. (2001). In the present investigation, the first condition was clearly not met in Experiment 1, where the biomass of live fine roots remained near 250 g m⁻² (about 110 g C m⁻²) before and after girdling (Fig. 5) and the respiration of roots < 3 mm was similar for control and girdled plots for at least 3.5 months (Fig. 6). The sustained biomass of fine roots and fine root respiration indicates that the *Eucalyptus* trees likely had large belowground stores of carbohydrates and that these pools sustained fine roots and fine root respiration, thereby accounting for substantial CO₂ efflux from the soil. Many *Eucalyptus* species (including those in these experiments) resprout vigorously after harvesting, also indicating substantial carbohydrate storage in roots (see Williams and Woinarski 1997). Edwards and Ross-Todd's (1979) girdling experiment also found little reduction in soil CO₂ efflux for girdled trees of species that resprout well after cutting.

The second condition may have been met in our study because girdling did not change live fine root biomass and, therefore, may not have increased the supply of

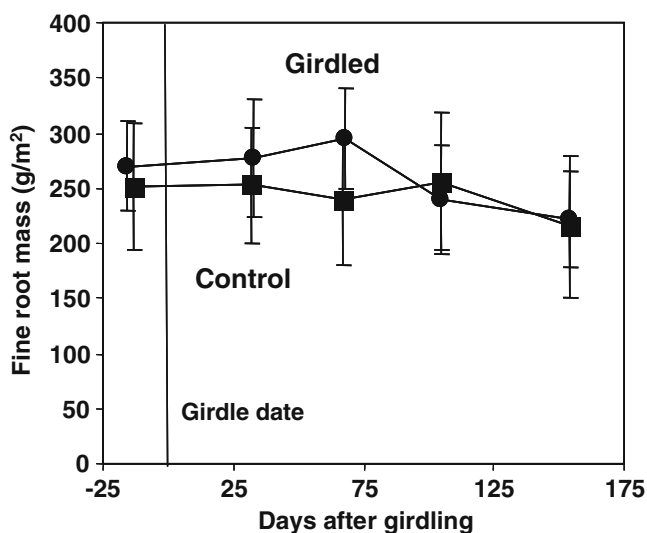


Fig. 5 Biomass of live fine roots in Experiment 1 did not respond to girdling for more than 150 days after girdling. Bars are standard errors of the means for four replicate plots

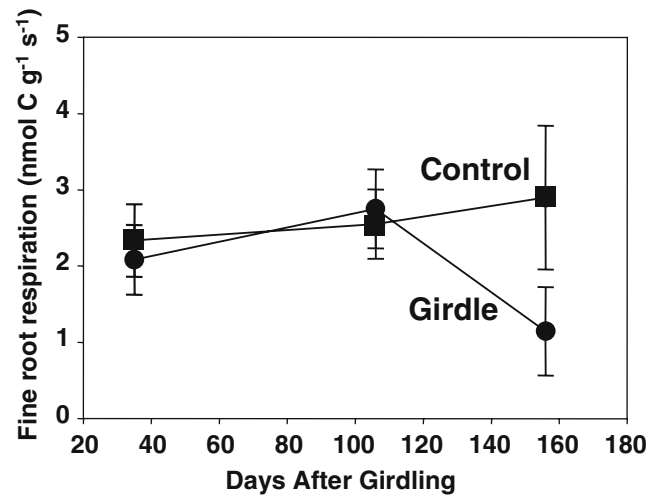


Fig. 6 Root respiration did not differ between the control and girdled plots after girdling for Experiment 1. Bars are standard errors of the means for four replicate plots

dead roots for heterotrophs to consume. Also, the decomposition of fine roots would not contribute much to soil CO₂ efflux because the biomass of the fine roots (125 g C m⁻²) was much lower than the CO₂ efflux over the 3-month evaluation period (325 g C m⁻²). This lack of a difference in the reduction in soil CO₂ efflux between the 50 and 100% treatments in Experiment 2 also suggests that one or both of these conditions may not be met in *Eucalyptus* plantations.

Both of our experiments showed much smaller proportional responses to girdling than the Scots pine stand described by Högberg et al. (2001), and the absolute response was also smaller. Soil CO₂ efflux from our *Eucalyptus* stands declined by 0.4–0.5 μmol C m⁻² s⁻¹, which is relatively small compared with declines of about 0.9–1.8 μmol C m⁻² s⁻¹ for the Swedish pine stand. Curiously, the extrapolation of our root respiration rates in Experiment 1 would give a root respiration flux of 0.60 μmol C m⁻² s⁻¹ or about 24% of the average soil CO₂ efflux over the post-girdling period (compared to the 16% contribution estimated by the girdling method). We suspect that this 0.60 μmol C m⁻² s⁻¹ estimate of root contribution is low because it excludes mycorrhizal respiration.

The availability of sufficient quantities of carbohydrates stored in *Eucalyptus* root systems and the ability of these trees to mobilize reserves and keep roots alive will probably prevent the girdling approach from being useful in separating plant and heterotrophic sources of CO₂ in these forests. We suggest more intensive characterization of belowground sources and sinks be included in other forests to establish the level of confidence warranted in using the girdling response to separate complex sources of soil CO₂ efflux.

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