

# **Studies of soil respiration in eucalypt forests of south east Australia**

**Vivien Edouard Marie de Rémy de Courcelles**

A thesis submitted in fulfilment of the requirements for the degree of  
Doctor of Philosophy

2014

Faculty of Agriculture and Environment  
The University of Sydney  
New South Wales  
Australia

## *Certificate of Originality*

This thesis is submitted to the University of Sydney in fulfilment of the requirements for the Doctor of Philosophy.

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Signature: Vivien de Rémy de Courcelles

Date: 30/07/2013

## *Acknowledgements*

I would like to thank my Supervisors, Professor Mark Adams and Bupinderpal Singh for giving me the opportunity to conduct this research.

My heartfelt thanks go to Dr Meaghan Jenkins for all her advices, the help in the field and the laboratory and most importantly being such a good friends during this journey.

I am very grateful to Dr Craig Barton and Buran Amidgi for always being present when I requested their help at HFE. Craig also provided the aboveground growth data that are part of Figure 2.1 in Chapter 2.

A lot of this work would not have been possible without the cooperation of Barry Aitcheson and Davel Dixon for letting me access and conduct my work on their wonderful property in the Snowy Mountains.

Neil Murdock and Mike Kemp were both great help in the field and in the workshop. The support and friendship of Chantelle Doyle, Andrew Merchant, Claudia Keitel, Jessica Obrien cannot go unnoticed.

For the risk of forgetting someone, I cannot name every person I met and tied links during these years at the University of Sydney and the University of New South Wales.

Finally a special thank you to my wife Emmanuelle who did not hesitate to accompany me in the field to help with the hardest part of my work and provided a

great support all along this journey... even toward the end when she felt I should wrap it up quickly! She also found the time to give birth to Justine, the cutest of all baby girls.

## *Abstract*

This thesis addresses gaps in knowledge of soil respiration in forests of south-east Australia. Soil respiration plays a major part in the cycle of carbon between soils - the biggest pool of terrestrial carbon - and the atmosphere. Despite its global significance, we have only a limited understanding of the magnitude and responses of soil respiration, and especially of its components, to abiotic (temperature, moisture, soil fertility) and biotic (photosynthesis, seasonality of belowground C allocation patterns and root growth, quality and quantity of above and belowground litter) controls. Furthermore, vegetation type may modulate the influences of these abiotic and biotic controls and with soil respiration research having been based mostly in the northern hemisphere, it is crucial that regional studies be conducted further afield. This thesis also considers the context of the current increase in atmospheric [CO<sub>2</sub>] and resulting predicted climate change that will directly or indirectly impact on soil respiration through extreme weather events, changes in the frequency and intensity of fires or increase in growth.

Using both field and laboratory based techniques I measured respiration from soils supporting a variety of Eucalypts. Elevated atmospheric [CO<sub>2</sub>] did not have an effect on rates of soil respiration in a *Eucalyptus saligna* plantation, contrary to usual findings. Drought on the other hand slowed rates of respiration, owing to a slowing of the transfer of photosynthates from leaves to roots. The impact of an increase in above-ground litter deposition, a possible consequence of extreme weather events, or continuous increase in primary production can be subdued by the nature and quality of the litter in *Eucalyptus pauciflora* woodlands. No effect was recorded in the field but ground litter added to soils in the laboratory triggered a response including a priming effect.

Root priming effect was also found to increase basal heterotrophic respiration by 54% on average in *Eucalyptus regnans*. The study on the contribution of roots to total soil respiration showed that it is necessary to use hybrid techniques to separate and estimate the contribution of components of soil respiration; in this thesis' case the use of collars and chambers in the field and respirometer in the laboratory was determinant in identifying root priming effect.

Great spatial variation in respiration rates was measured both in the simple ecosystem of a *Eucalyptus saligna* plantation and as a result of fire disturbance at the Messmate 1 site supporting *Eucalyptus obliqua* and *Eucalyptus radiata*.

Finally, a synthesis of the results of the whole thesis considered the effect of soil temperature on soil respiration and showed that contrary to what is commonly agreed by the  $Q_{10}$  model, respiration rates reached a plateau for temperatures between 16°C to 23°C.

## *Table of contents*

<b>Certificate of Originality</b>	<b>i</b>
<b>Acknowledgements</b>	<b>ii</b>
<b>Abstract</b>	<b>iv</b>
<b>Table of contents</b>	<b>ix</b>
<b>List of figures</b>	<b>xiii</b>
<b>List of tables</b>	<b>xvii</b>
<b>Chapter 1. Introduction. Soil C, sources of respired CO<sub>2</sub>, and approaches to measurement</b>	<b>1</b>
1.1 Introduction	1
1.2 Isolation and quantification of the components of soil respiration	3
1.3 Regulation of soil respiration	8
1.3.1 <i>Photosynthesis and carbon allocation</i>	8
1.3.2 <i>Temperature</i>	9
1.3.3 <i>Soil moisture</i>	11
1.3.4 <i>Nitrogen availability</i>	12
1.4 Responses of soil respiration to future global change scenarios	13
1.4.1 <i>Increased atmospheric CO<sub>2</sub></i>	14
1.4.2 <i>Climate warming</i>	16
1.4.3 <i>Precipitation extremes</i>	18
1.4.5 <i>Fire</i>	21
1.5 Soil respiration in a changing world - interactions	24
1.6 Objectives	26
<b>Chapter 2. Effects of availability of soil water and atmospheric CO<sub>2</sub> on soil respiration in a Eucalyptus saligna plantation</b>	<b>29</b>
2.1 Introduction	29
2.2 Materials and methods	32
2.2.1 <i>Site description</i>	32
2.2.2 <i>Belowground respiration measurements</i>	35
2.2.3 <i>Statistical analysis</i>	37
2.3 Results	37

2.3.1	<i>Soil respiration and aboveground biomass</i>	37
2.3.2	<i>Irrigation treatment</i>	39
2.3.3	<i>Rewatering effect</i>	40
2.3.4	<i>Elevated [CO<sub>2</sub>] treatment</i>	41
2.3.5	<i>Interaction between treatments</i>	42
2.3.6	<i>Soil temperature and moisture</i>	42
2.3.7	<i>Partitioning of components of respiration and the 'WTC effect'</i>	45
2.3.8	<i>Root growth</i>	46
2.4	Discussion	47
2.4.1	<i>Irrigation treatment</i>	47
2.4.2	<i>Elevated [CO<sub>2</sub>] effect</i>	50
2.4.3	<i>Soil respiration partitioning</i>	52
2.4.4	<i>Whole-tree chamber effect</i>	53
2.4.5	<i>Spatial variability in soil respiration</i>	54
2.5	Conclusion	55
 <b>Chapter 3. The tracing of carbon through trees and soil from leaves to atmosphere: a pulse labelling experiment</b>		<b>56</b>
3.1	Introduction	56
3.2	Materials and methods	58
3.2.1	<i>Field site and plant and gas sampling</i>	58
3.2.2	<i>Isotope analysis and theory</i>	62
3.3	Results	64
3.4	Discussion	73
3.5	Conclusion	76
 <b>Chapter 4. The recovery of soil respiration post fire: examples of two sites in South East Australia.</b>		<b>78</b>
4.1	Introduction	78
4.2	Materials and Methods	81
4.2.1	<i>Mount Disappointment State Forest</i>	81
4.2.2	<i>Snowy Plains</i>	86
4.2.3	<i>Statistical analysis</i>	88
4.3	Results	89
4.3.1	<i>Belowground respiration</i>	89
4.3.2	<i>Effect of soil moisture</i>	92



4.3.3 <i>Soil temperature effect</i>	94
4.3.4 <i>Cumulative soil respiration</i>	95
4.3.5 <i>Weather</i>	97
4.3.6 <i>Microbial biomass</i>	97
4.4 Discussion	99
4.5 Conclusion	103
<b>Chapter 5. Contributions of tree roots to soil respiration: field and laboratory studies</b>	<b>104</b>
5.1 Introduction	104
5.2 Materials and Method	106
5.2.1 <i>Site description</i>	106
5.2.2 <i>Trenching and soil respiration measurements</i>	107
5.2.3 <i>Statistical analysis</i>	109
5.3 Results	111
5.3.1 <i>Field experiment</i>	111
5.3.2 <i>Laboratory experiment</i>	113
5.4 Discussion	117
5.5 Conclusion	120
<b>Chapter 6. Increase of litter input to soil and belowground respiration</b>	<b>122</b>
6.1 Introduction	122
6.2 Materials and methods	125
6.2.1 <i>Field experiment</i>	126
6.2.2 <i>Laboratory experiment</i>	128
6.2.3 <i>Statistical analysis</i>	129
6.3 Results	130
6.3.1 <i>Litter effect</i>	130
6.3.2 <i>Cumulative respiration and priming effect</i>	137
6.3.3 <i>Respiratory quotients</i>	139
6.4 Discussion	140
6.4.1 <i>Litter addition and heterotrophic respiration</i>	140
6.4.2 <i>Priming effect</i>	141
6.4.3 <i>Field study</i>	143
6.4.4 <i>Temperature effect</i>	144
6.5 Conclusion	145

<b>Chapter 7. Conclusion</b>	<b>146</b>
7.1 Techniques for measuring soil respiration	146
7.2 Autotroph-heterotroph continuum	149
7.3 Regional specificity	151
7.4 Fire and Australian ecosystems	156
7.5 Conclusion	158
<b>References</b>	<b>161</b>

## *List of figures*

### **Chapter 1.**

Figure 1.1. Soil respiratory components, autotrophic (root, rhizmicrobial) and “true” heterotrophic respiration, arranged according to their dependency on substrate type (with their turnover times ranging from days for recent photosynthates to months to decades for complex forms of organic matter in soil), belowground carbon allocation supply and environmental controls (soil temperature and water availability). Nitrogen availability in soil may equally influence both autotrophic and heterotrophic components of soil respiration. (From Singh et al. 2011) 3

### **Chapter 2.**

Figure 2.1. Rates of belowground respiration and aboveground biomass of the *Eucalyptus saligna* at HFE in (a) Irrigated-ambient [CO<sub>2</sub>], (b) Droughted-elevated [CO<sub>2</sub>], (c) Droughted-ambient [CO<sub>2</sub>], (d) Irrigated-elevated [CO<sub>2</sub>] whole tree chambers (WTC). Results are averaged over three replicates of WTC. Aboveground biomass data provided by Craig Barton. 38

Figure 2.2. Time series of soil respiration measured at the Hawkesburry Forest Experiment (HFE) between March 2008 and February 2008 plotted against the watering treatment. Each line is the mean of six replicates across three ambient WTC and three elevated [CO<sub>2</sub>] WTC. Stars show a significant difference ( $P < 0.05$ ) in soil respiration calculated with two-way ANOVA. 40

Figure 2.3. Time series of soil respiration following rewatering on 4 March 2009. Soil respiration was measured using the under-floor space of the WTC as a static chamber. Mean of six replicates across three ambient WTC and three elevated WTC subjected to drought treatment (receiving no water) or irrigated treatment. 41

Figure 2.4. Time series of soil respiration at HFE averaged over six control plots, six elevated [CO<sub>2</sub>] WTC and six ambient [CO<sub>2</sub>] WTC across irrigation and drought treatment. Standard error bars are displayed in the negative direction. 42

Figure 2.5. Soil microbial biomass (0-10 cm depth) from the ambient WTC before and up to 13 days after the 5 March 2009 rewatering. 45

Figure 2.6. Root length density (a) and root weight (b) recorded with a mini-rhizotron on four dates Results are averaged over the four dates and reported as mean across three replicates for each [CO<sub>2</sub>]-irrigation treatment combination and over the three control for each irrigation treatment. Data provided by Tarryn Turnbull. 47

### **Chapter 3.**

Figure 3.1. Draw-down after introduction of 5 l of  $^{13}\text{CO}_2$  in the whole tree chambers at HFE in the droughted and irrigated treatments. 66

Figure 3.2.  $\delta^{13}\text{C}$  of *E. saligna* leaves during the pulse chase period. The vertical dotted line represents the pulse labeling and the horizontal dashed line is the pre-pulse value  $\delta^{13}\text{C}$  for each tree. Light markers indicate droughted WTC, dark markers are irrigated WTC. 67

Figure 3.3. Evolution of the  $\delta^{13}\text{C}$  values of soil respiration ( $\delta^{13}\text{C}_{\text{RS}}$ ) of each of the labeled WTC during the 26 day pulse-chase. 68

Figure 3.4. Evolution of  $\delta^{13}\text{C}_{\text{roots}}$  in the root tips collected at three different depths for each WTC and treatment during the 34 day pulse chase. 71

Figure 3.5. Evolution of  $\delta^{13}\text{C}_{\text{roots}}$  in the fine roots (<1 mm) and coarse roots (>1 mm) collected at three different depths during the 34 day pulse chase. Results are averaged over three WTC for each treatment. 72

#### Chapter 4.

Figure 4.1. Mapping of the moss beds at the Messmate 1 and Mountain Ash sites of the Mt Disappointment State forest. 84

Figure 4.2. Rates of soil respiration at the three sites of the Mount Disappointment State forest 90

Figure 4.3. Rates of soil respiration at the Messmate 1 site of the Mt Disappointment state forest. \* indicates significant difference (t test,  $P < 0.05$ ) 91

Figure 4.4. Rates of soil respiration at the Snowy Plains. Rates are averaged over the three woodland sites. 92

Figure 4.5. Volumetric water content at all sites of Mt Disappointment (a) and the Snowy Plains (b) 94

Figure 4.6. Cumulative soil respiration at the three sites of the Mount Disappointment state forest (a) and the Snowy Plains (b) 96

Figure 4.7. Soil microbial biomass from chloroform fumigation and 0.5M  $\text{K}_2\text{SO}_4$  extraction at (a) Mt Disappointment and (b) the Snowy Plains 98

#### Chapter 5.

Figure 5.1. Rates of belowground respiration measured in situ for the trenched and un-trenched plots of the Mountain Ash site of the Mt Disappointment state forest. Star symbol indicates significant difference in respiration rates (t-test,  $P < 0.05$ ). 111

Figure 5.2. Respective proportion of autotrophic (Ra) and heterotrophic (Rh) soil respiration as calculated from the mean of the five trenched plots and the mean of the five un-trenched plots 112

Figure 5.3. Rates of heterotrophic respiration measured at 20°C with the respirometer for the trenched and un-trenched plots. Rates are averaged over the measurements of five soil samples for each of the trenched and un-trenched plots. 115

Figure 5.4. Microbial biomass of trenched as a function of un-trenched plots. Each point is calculated from Chloroform fumigation and K<sub>2</sub>SO<sub>4</sub> extraction on three replicates of soil samples collected at each of the five trenched and un-trenched plots. 116

## Chapter 6.

Figure 6.1. Soil respiration from the controls, no litter and double litter plots measured on site at the Snowy Plain. 131

Figure 6.2. Soil temperature (a) and soil volumetric water content (b) measured in the field on the control (standard litter), double litter and no litter plots. 132

Figure 6.3. Microbial biomass and labile carbon measured after Chloroform fumigation and K<sub>2</sub>SO<sub>4</sub> extraction from soil samples collected in March 2011, one year after manipulation. 133

Figure 6.4. Heterotrophic soil respiration measured on the respirometer in the laboratory for woodland (a) and grassland (b) soils. Litter amendments are 0.7 g woodland litter/100g DW woodland soil and 0.16 g grassland litter/100g DW grassland soil. Double litter amendments are addition of 1.4 g woodland litter/100g DW woodland soil and 0.32 g grassland litter/100g DW grassland soil. “\*” indicates significant difference to the non amended soils ( $P < 0.05$ ) 135

Figure 6.5. Heterotrophic soil respiration measured in the laboratory at 5, 10, 15, 20 and 25°C. on day 16 of litter incubation with fitted curves. Litter amendments are 0.7 g woodland litter/100g DW woodland soil and 0.16 g grassland litter/100g DW grassland soil. Double litter amendments are addition of 1.4 g woodland litter/100g DW woodland soil and 0.32 g grassland litter/100g DW grassland soil. 136

Figure 6.6. Cumulative heterotrophic soil respiration from woodland (a) and grassland soils (b). Litter amendments are 0.7 g woodland litter/100g DW woodland soil and 0.16 g grassland litter/100g DW grassland soil. Double litter amendments are addition of 1.4 g woodland litter/100g DW woodland soil and 0.32 g grassland litter/100g DW grassland soil. 138

Figure 6.7. Respiratory quotient calculated as the number of moles of CO<sub>2</sub> released divided by the number of moles of O<sub>2</sub> absorbed during respiration measurements of the grassland soils (a) and woodland soils (b) with no litter, single addition and double addition of litter. 139

## Chapter 7.

Figure 7.1. Temperature dependence of soil respiration across all experimental sites presented in previous chapter: raw soil respiration data (a) and normalized data (relative to the maximum respiration rates observed at each site) (b). The curve drawn in (a) is not fitted to the data. 153

## *List of tables*

### **Chapter 1.**

Table 1.1. Estimates of the contribution of autotrophic respiration to total soil respiration based on some novel partitioning methods. 5

### **Chapter 2.**

Table 2.1. Results of two-way ANOVA analysis of the effect of the irrigation treatment, [CO<sub>2</sub>] treatment and their combination on soil respiration within WTC. WTC effect is the result of ANOVA analysis of soil respiration from ambient WTC and control plots. All results in bold are significant ( $P < 0.05$ ). 39

Table 2.2.  $P$  values per treatment and for each treatment from linear regression analysis between soil temperature and soil respiration and soil volumetric water content and soil respiration. 45

### **Chapter 3.**

Table 3.1. Amount of <sup>13</sup>CO<sub>2</sub> taken up by trees during pulse labeling and recovered into soil respiration during pulse chase after 9723 mg of <sup>13</sup>CO<sub>2</sub> were introduced in each WTC. Result in mg of <sup>13</sup>CO<sub>2</sub> plus/minus standard error 65

Table 3.2. Mean residence time and half life of the <sup>13</sup>C label in the soil CO<sub>2</sub> effluxes as calculated by equation 3 69

### **Chapter 4.**

Table 4.1. Coefficients of equations generated by general linear model of soil respiration as a function of soil volumetric water content and temperatures at Mt Disappointment and the Snowy Plains. All models are significant at  $P < 0.01$  95

### **Chapter 5.**

Table 5.1. Soil characteristics at all soil sampling dates. Mean calculated from five samples from each of the un-trenched (U) and trenched (T) plots. Microbial biomass and labile carbon are given with standard error. Both soil temperature (Temp) and gravimetric water content (GWC) were measured in the field when sampling. 109

Table 5.2. Rates of heterotrophic respiration ( $\mu\text{g CO}_2\text{-C g dry soil}^{-1} \text{ hour}^{-1}$ ) measured on the respirometer between 5°C and 25°C. Results with standard errors are averaged

over measurements performed on five samples taken from the trenched plots ( $R_t$ ) and five samples taken from the un-trenched plots ( $R_u$ ). 114

Table 5.3.  $Q_{10}$  values calculated from the respirometer measurements of heterotrophic respiration between 5°C and 25°C and respiratory quotient calculated at 20°C. 117

## **Chapter 6.**

Table 6.1. Amount of elements added to 100 g dry weight of grassland and woodland soils when 0.16 g of grassland litter (GL) and 0.7g of woodland litter (WL) were added to either soil. 130

Table 6.2. R square and *P*-value for linear regression of rate of heterotrophic soil respiration measured in the laboratory at 5, 10, 15, 20 and 25°C on day 16 of litter incubation and for grassland and woodland soils with various amount of litter addition. 137

## **Chapter 7.**

Table 7.1. Rates of soil respiration measured around the world in various forest ecosystems 152

Table 7.2. Examples of mega-fires in the world. From Attiwill and Binkley, 2013. 160



## *Chapter 1*

### *Introduction*

#### *Soil C, sources of respired CO<sub>2</sub>, and approaches to measurement*

##### *1.1 Introduction*

Soil respiration is a major force in the regulation of carbon (C) cycling and climate in the earth system (Luo and Zhou 2006). Soil respiration releases carbon dioxide (CO<sub>2</sub>) from soil to the atmosphere via the combined activity of (i) roots (autotrophic root respiration) and associated microorganisms, mainly respiring the recently-assimilated C by plants (rhizomicrobial respiration), and (ii) micro- and macroorganisms decomposing litter and organic matter (humus) in soil, referred to as heterotrophic respiration (see Figure 1.1 and details below; Högberg et al. 2005). After photosynthesis, soil respiration is the second largest C flux in most terrestrial ecosystems, and may account for ~70% of total ecosystem respiration on annual basis (Kuzyakov and Larionova 2005). Globally, soil respiration emits 98±12 Pg CO<sub>2</sub>-C yr<sup>-1</sup> to the atmosphere (Raich et al. 2002). This flux is approximately an order of magnitude larger than the current annual anthropogenic CO<sub>2</sub>-C emissions from fossil fuel combustion (Boden et al. 2010). Soil respiration provides the pathway between the organic C reserves in the soils and the atmosphere. Organic C reserves within the surface soil horizons (to the depth of 1 m) of terrestrial ecosystems are huge (1576 Pg) and exceed the combined total C in the aboveground biomass (550 Pg) and the atmosphere (750 Pg, Schlesinger and Andrews 2000). Increased storage of carbon in world soils could help offset anthropogenic emissions of CO<sub>2</sub> and this is

rapidly becoming a priority focus for global research and policy. In similar but opposite fashion, even a small change in release of CO<sub>2</sub> from soil via soil respiratory processes would have a profound impact on the atmospheric CO<sub>2</sub> budget at regional and global scales (Rustad et al. 2000). Any processes, climate perturbations, or management practices that decrease organic C inputs to or enhance outputs from soil are likely to have adverse implications for soil health and climate change (Bond-Lamberty and Thomson 2010).

Despite this clear global significance, we have only a limited understanding of the magnitude and responses of soil respiration, and especially of its components, to abiotic (temperature, moisture, soil fertility) and biotic (photosynthesis, seasonality of belowground carbon allocation patterns and root growth, quality and quantity of above and belowground litter) controls. Furthermore, soil respiration is generally faster (~ 20%) in grasslands than forest stands, and slower (~ 10%) in coniferous forests than adjacent broad-leaved forests, under similar edaphic and climatic conditions, demonstrating that vegetation type may modulate the influences of abiotic and biotic controls on soil respiration (Raich and Tufekcioglu 2000). Critical elements in understanding mechanisms responsible for belowground C cycling and whether a soil will become a future CO<sub>2</sub> source or sink are: (i) quantification of individual soil respiratory components, and (ii) assessment of their responses to different environmental and plant factors (e.g. temperature, moisture, nitrogen (N) availability, seasonality of carbon allocation).

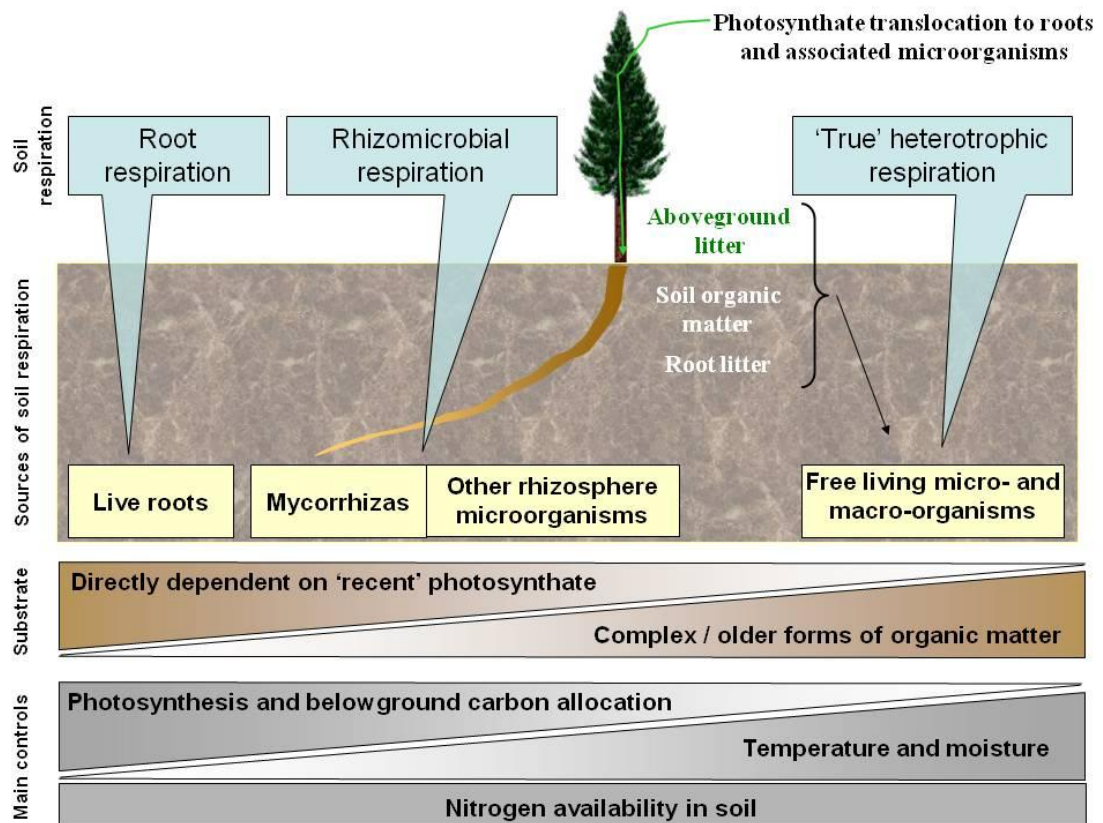


Figure 1.1.

Soil respiratory components, autotrophic (root, rhizomicrobial) and “true” heterotrophic respiration, arranged according to their dependency on substrate type (with their turnover times ranging from days for recent photosynthates to months to decades for complex forms of organic matter in soil), belowground carbon allocation supply and environmental controls (soil temperature and water availability). Nitrogen availability in soil may equally influence both autotrophic and heterotrophic components of soil respiration. (From Singh et al. 2011)

### ***1.2 Isolation and quantification of the components of soil respiration***

Because root respiration and “rhizo-microbial” respiration are extremely difficult to measure separately, they are often grouped as autotrophic respiration (Cisneros-Dozal et al. 2006, Hogberg et al. 2001, Kuzyakov 2006). Nevertheless, rhizomicrobial respiration results mainly from the activity of mycorrhizal fungi and other rhizosphere microorganisms growing on plant assimilated C, and should, taxonomically, be classified as heterotrophic respiration. In recognition of the

difficulty of separating autotrophic and heterotrophic respiration under natural (field) conditions, Högberg et al. (2005) suggested considering an autotroph-heterotroph continuum. According to this autotroph-heterotroph continuum, autotrophic respiration includes the respiration of roots and associated microorganisms that are directly dependent on recent plant-assimilated carbon for their activity. The “true” heterotrophic respiration however includes respiration from free-living micro- and macro-organisms oxidising plant litter, and inherent soil organic matter, containing more complex organic molecules than recent plant assimilates (Cisneros-Dozal et al. 2006).

Detailed reviews of the methods used to partition soil respiratory components have been presented in Hanson et al. (2000) and Högberg et al. (2005). In summary, the methods employed can be grouped into four broad categories: (1) integration of physically-disintegrated respiratory components (root, leaf litter, soil organic matter, etc); (2) exclusion of live roots from soil monoliths via root trenching, with the assumption that severed roots disappear by decomposition within a short time; (3) the use of non-invasive stable or radioactive isotopes that rely on different ‘isotopic signatures’ of CO<sub>2</sub> derived from living roots and soil organic matter; and, (4) stem girdling in forest ecosystems (involving instantaneous termination of photosynthetic C flow to roots and associated microorganisms without affecting micro-climate, at least initially, Högberg et al. 2005). More recently, mycorrhizal mesh-collar chambers have been employed *in situ* for isolation of the contributions of root, extraradical ectomycorrhizal, and soil heterotrophic respiration to total soil respiration in a young Lodgepole pine forest (Heinemeyer et al. 2007). This technique has been tested in a limited range of ecosystems.

Table 1.1.

Estimates of the contribution of autotrophic respiration to total soil respiration based on some novel partitioning methods.

Biome	Vegetation type (age in years)	Approach	Ra/Rs*	References
Boreal	Scots pine forest (45–55)	Girdling	0.54	Högberg et al. (2001)
	Scots pine forest (45–55)	Girdling	0.65 (2 <sup>nd</sup> year)	Bhupinderpal-Singh et al. (2003)
	Norway spruce forest (40)	Girdling	0.40 / 0.60§	Olsson et al. (2005)
	Norway spruce-dominated forest (120)	Girdling	0.53	Högberg et al. (2009)
	Scots pine-dominated forest (150)	<sup>13</sup> C of respired CO <sub>2</sub> in response to seasonal variations in air relative humidity	0.65	Ekblad and Högberg (2001)
Temperate	Norway spruce forest (35)	Girdling	0.50	Subke et al. (2004)
	European Chestnut (57)	Girdling	0.36	Frey et al. (2006)
	Mixed deciduous forest (~60)	Radiocarbon	0.66	Gaudinski et al. (2000)
	Mixed deciduous forest (~60)	Radiocarbon	0.71 / 0.80¶	Borken et al. (2006)
	Tall grass prairie (na)	Clipping ± shading	0.30	Wan and Luo (2003)
	Montane spruce-dominated forest (130)	Root trenching	0.44	Díaz-Pinés et al. (2010)
	Douglas-fir forest (55)	Root trenching	0.39	Jassal and Black (2006)
	Loblolly pine forest (15)	<sup>13</sup> C-depleted CO <sub>2</sub> (in Free-Air Carbon Dioxide Enrichment plots)	0.55	Andrews et al. (1999)
Tropical	Eucalyptus plantation (2)	<sup>13</sup> C (from C <sub>4</sub> to C <sub>3</sub> vegetation change)	0.68 / 0.73§	Giardina et al. (2004)
	Eucalyptus plantation (6.5)	Girdling	0.24	Binkley et al. (2006)
	Old moist lowland forest (na)	Root trenching	0.38	Sayer and Tanner (2010)
	Semi-arid perennial grasses (na)	Radiocarbon	0.74	Carbone et al. (2008)
	Semi-arid shrubs (na)	Radiocarbon	0.81	Carbone et al. (2008)
	Savannah (na)	<sup>13</sup> C (from C <sub>4</sub> to C <sub>3</sub> vegetation gradients)	0.46	Millard et al. (2008)

\*Maximal contributions of root respiration (Ra) to total soil respiration (Rs), either on day, seasonal or annual time steps, as reported by the authors are included here. 'na' means not available.

§from fertilised/non-fertilised treatments, respectively.

¶from control/throughfall exclusion treatments, respectively

Estimates of the contribution of autotrophic or heterotrophic respiration to total soil respiration vary widely depending on the type of ecosystem studied and the methods used to partition the fluxes (Hanson et al. 2000, Subke et al. 2006). Contributions of soil respiratory components are also influenced by the seasonality of plant C allocation as well as by climatic controls on microbial and/or root activities (Högberg et al. 2005, Subke et al. 2006). In recent years, despite variations in biomes and ecosystems studied, the development and careful application of some of the more novel techniques, such as tree-girdling, grass clipping ± shading, root trenching, and non-invasive C-isotope based approaches have helped refine estimates (Table 1.1). For example, using a tree girdling approach autotrophic respiration in a boreal forest was estimated to contribute between 50 and 65% of total soil respiration (Bhupinderpal-Singh et al. 2003, Hogberg et al. 2001). Högberg et al. (2009) reported a maximum mean contribution of autotrophic respiration of *ca.* 56% for unfertilised boreal forests and *ca.* 44% for temperate forests. Contributions of autotrophic respiration in a subtropical eucalyptus forest were much smaller (24%) than those reported by Högberg et al. (2009), and this was attributed to continuous respiration of large root starch reserves for up to several months following tree girdling (Binkley et al. 2006). In the same way as tree girdling, a grass clipping ± shading approach has also reasonably separated soil respiratory components, such as root plus rhizomicrobial (30%), aboveground litter (14%) and belowground litter plus soil organic matter (56%), in a tall grass prairie. Novel root trenching approaches that eliminate or account for methodological artefacts associated with classical long-term trenching experiments seemingly allow robust partitioning of autotrophic and heterotrophic components of soil respiration (Diaz-Pines et al. 2010, Jassal and Black 2006, Sayer and Tanner 2010). Furthermore, non-invasive C isotope

methods involve fewer methodological artefacts than most other partitioning methods (Högberg et al. 2005, Subke et al. 2006), and have consistently reduced the variability attributed to estimates of the contributions of soil respiratory components to total soil CO<sub>2</sub> efflux (Paterson et al. 2009).

Each component of soil respiration returns C to the atmosphere on different time scales, with rapid C cycling associated with root/rhizosphere respiration (days to months), followed by litter C decomposition (years), and then inherent soil organic matter decomposition (decades to centuries, Cisneros-Dozal et al. 2006, Kuzyakov and Larionova 2005). Increases in root respiration may not directly affect soil carbon storage because root respiration is closely linked to carbon uptake by photosynthesis and, obviously, to the proportion of recently fixed carbon that is distributed to the belowground parts of plants whereas increases in heterotrophic respiration may lead to significant reductions in soil C stocks. The relatively slow turnover rate of litter and soil organic matter makes heterotrophic respiration an important component of soil respiration with profound implications for long-term storage of organic C in soil.

The rate at which carbon is ‘allocated’ belowground and then exuded into the soil, can indirectly affect soil C turnover, through stimulation or the so-called ‘priming’ of microbial respiration (Kuzyakov 2002, Kuzyakov and Cheng 2001). ‘Priming effects’ were named by Bingeman et al. (1953) and is defined as “strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil” (Kuzyakov et al. 2000). Soil respiration may be primed by the addition of substrates through litter and root decomposition (Crow et al. 2009, Hamer

and Marschner 2005, Nottingham et al. 2009, Prevost-Boure et al. 2010, Sulzman et al. 2005), or an increase in root exudation or the colonisation of soils by roots (Cheng and Kuzyakov 2005, Dijkstra and Cheng 2007a, Zhu and Cheng 2011). Priming effects may be positive, leading to an increase in belowground respiration that is greater than the combined CO<sub>2</sub> effluxes of untreated soil and that from the substrate turn over or root activity, or negative, resulting in slower rates of respiration (Blagodatskaya and Kuzyakov 2008, Kuzyakov et al. 2000). The direction of priming (ie positive or negative) is directly dependent on the C:N ratio of the active SOM pool as well as the nutrient composition of soils (Kuzyakov et al. 2000) and the amount of organic carbon added relative to the carbon contained in soil micro organisms (Blagodatskaya and Kuzyakov 2008). The extent of rhizosphere priming is governed by plant species (Dijkstra and Cheng 2007a) and phenology (Cheng et al. 2003), atmospheric concentration in CO<sub>2</sub> (Cardon 1996), plant biomass (Dijkstra et al. 2006), nutrient status of soil (Liljeroth et al. 1994) and soil moisture (Dijkstra and Cheng 2007b). Many of these influences are predicted to change under future climate scenarios.

### ***1.3 Regulation of soil respiration***

#### *1.3.1 Photosynthesis and carbon allocation*

Girdling, clipping and shading can be used as experimental treatments that slow or terminate carbon supply from aboveground plant tissues to roots (Bhupinderpal-Singh et al. 2003, Ekblad et al. 2005, Hogberg et al. 2001, Kuzyakov and Cheng 2001, Wan and Luo 2003) allowing researchers to examine their effect on belowground processes. Pulse-labelling has been used to trace the flux of recently-



fixed C through the plant-microbe-soil continuum (Hogberg et al. 2008). Combining treatments and techniques has allowed demonstration of a direct and rapid link between the supply of photosynthates and autotrophic components of soil respiration. These studies clearly show that photosynthesis and patterns of carbon allocation are major controls on root respiration. Globally, soil respiration is positively related to aboveground net primary production, and consequently to litter production (Raich and Schlesinger 1992). For heterotrophic soil respiration, substrates are supplied via root exudates, leaf litter, root litter and native soil organic matter. Root exudates are mostly highly labile while leaf and root litter are rather less available and native soil organic matter is usually the least available. Manipulations of litterfall suggest a clear relationship with soil respiration (Fontaine et al. 2004, Sayer et al. 2007, Sulzman et al. 2005) and litterfall phenology helps determine soil respiration patterns on an annual basis (Deforest et al. 2006). Factors that limit photosynthesis or the supply of photosynthates to roots, for example, irradiation, water availability, soil fertility, and aboveground herbivory, or the accessibility and availability to microbial enzymes of soil organic matter (e.g. aggregation, water availability, substrate quality), serve as confounding factors and may mask the effect of rates of supply of substrates for respiration. It is important to note, but difficult to isolate, the influence of the supply of substrate from such confounding factors. Any time lag between photosynthesis and root and root-derived respiration must also be considered when interpreting autotrophic respiration (Kuzyakov and Gavrichkova 2010).

### *1.3.2 Temperature*

In global models, temperature is widely considered the most important abiotic control of soil respiration (Cox et al. 2000, Falge et al. 2002, Fang and Moncrieff 2001). In these models, the relationship of soil respiration to temperature is often described by  $Q_{10}$ -based formulations and applied at local, regional and even global scales (e.g. Cox et al. 2000, Falge et al. 2002, Fang and Moncrieff 2001). Often  $Q_{10}$  is assumed = ~2, i.e. the respiration rate doubles for every 10°C increase in temperature, and rather too rarely do authors acknowledge differences in short- and long-term responses and acclimation of soil respiration to changes in temperature (e.g. Atkin et al. 2000, Larigauderie and Korner 1995). Because  $Q_{10}$  of soil respiration is often derived via seasonal variations in temperature, it is difficult to separate the temperature effect from other seasonally variable influences such as carbon allocation to roots, solar radiation and moisture. Some studies have suggested different seasonally-derived  $Q_{10}$  values for root and heterotrophic respiration, with root respiration being twice as sensitive ( $Q_{10}$  ~4) as heterotrophic respiration ( $Q_{10}$  ~2) (Boone et al. 1998, Epron et al. 2001). This suggestion has been criticised because of the lack of consideration given to such confounding variables (e.g. seasonality of belowground carbon allocation and root growth) in increasing  $Q_{10}$  of root respiration (Bhupinderpal-Singh et al. 2003, Hogberg et al. 2001, Ruehr and Buchmann 2010, Schindlbacher et al. 2009). In fact, some studies demonstrate a lack of response of root and/or mycorrhizal respiration to temperature (Heinemeyer et al. 2007, Högberg et al. 2005), especially during periods when C allocation remained more-or-less constant (Bhupinderpal-Singh et al. 2003).

While soil respiration and soil and/or air temperatures are frequently related (Hogberg et al. 2009), temperature cannot automatically be considered the most

important determinant of root respiration that seems substrate-limited for long periods (Schindlbacher et al. 2009). Similarly, while temperature is a major regulator of the decomposition of litter and soil organic matter (Schindlbacher et al. 2009), the accessibility and supply of organic substrates to microbial enzymes in soil play key roles in limiting heterotrophic respiration (Davidson and Janssens 2006, Davidson et al. 2006, Schindlbacher et al. 2009).

### *1.3.3 Soil moisture*

The availability of moisture clearly limits many biological processes in soils, including respiration. The activity of both root and heterotrophic respiratory components are usually reduced when soils are either very wet or very dry. When very dry, the activity of heterotrophs can be limited by the rate of diffusion of extracellular enzymes and soluble C substrates. This can potentially lead to dormancy and/or death of microorganisms as well as a reduction in microbial mobility (Orchard and Cook 1983). Soil fungi are generally better adapted to water stress than bacteria or microfauna in soil (Swift et al. 1979). On the other hand, gas exchange and soil oxygen concentrations are usually reduced in very wet soils, including at sites of microbial and root activity.

As with temperature, seasonal covariance of photosynthetic rate, litterfall, precipitation and root and microbial activity, make highly difficult interpretation of the effect of soil moisture on soil respiration (Jassal et al. 2008). Nevertheless, across a range of ecosystems at moderate temperatures (e.g. > 16 °C, Almagro et al. 2009),

soil moisture emerges as a main driver of soil respiration, especially in the range < 20% volumetric soil moisture content (Almagro et al. 2009, Jassal et al. 2008).

#### *1.3.4 Nitrogen availability*

Soil N availability indirectly affects soil respiration and its components by influencing plant growth and productivity, belowground carbon allocation, fine root growth, litter quantity and quality, microbial enzyme activity, composition of decomposer community, and decomposability of recalcitrant organic matter in soil (Janssens et al. 2010). Productive, nitrogen-rich soils usually support faster rates of plant growth and growth respiration is increased commensurately. Increased plant growth usually results in greater annual litterfall, thereby providing greater availability of substrates for heterotrophic activity. On the other hand, rates of fine root production, including mycorrhizal colonisation of roots, are often less under high-N conditions and both root and heterotrophic respiration may also be reduced (Janssens et al. 2010). The reverse trend might be expected in N-poor soil – greater investment by plants of fixed energy (photosynthate) into the growth of fine roots and mycorrhizas - leading to increased rates of respiration.

The quality of litter and soil C, and N availability and its interaction with organic C, help regulate decomposition of organic matter in soil. There may be only weak responses to the application of N by plant litter with wide C-to-N (>30) and lignin-to-N ratios (as found in many forest ecosystems) owing to enhanced formation of recalcitrant compounds (Janssens et al. 2010, Knorr et al. 2005). In a major review, Fog (1988) hypothesized the reasons for slower decomposition of plant materials

under optimal N availability were: (a) formation of additional recalcitrant compounds (such as polyphenol-organic N complexes, and/or (b) inhibition of appropriate growth conditions for certain fungi (white rot) with lignolytic enzyme synthesis capabilities.

#### ***1.4 Responses of soil respiration to future global change scenarios***

Current atmospheric CO<sub>2</sub> concentrations are ~110 ppm above preindustrial concentrations of 280 ppm. Atmospheric CO<sub>2</sub> concentrations are predicted to increase further to >550 ppm by 2100 (Forster et al. 2007). These increases as well as those of other greenhouse gases in the Earth's troposphere, are contributing to global warming and possibly to changes in patterns of precipitation (Forster et al. 2007). It has been estimated that global mean surface temperature will rise by 1.6 to 6.4 °C by 2100 (Forster et al. 2007), while the frequency of more intense rainfall events may increase by 5% - 10% per °C of warming, similar to the rate of increase in atmospheric water vapour content (Allison et al. 2009). Intensification of hydrological cycles seems likely to be accompanied by increased precipitation extremes, including heavy precipitation in already-wet areas and increased drought in already-dry areas (Allison et al. 2009). For example, subpolar and polar regions are likely to experience increases in precipitation whereas an opposite trend (e.g. longer dry spells followed by heavier but shorter precipitation events) is expected in (sub)-tropical regions (Solomon et al. 2007) such as Australia. These drier and hotter conditions predicted by climate models in the tropical regions will in all probability contribute to amplify the number, intensity and extent of fires in these parts of the world (Flannigan and Vanwagner 1991, Stocks et al. 1998, Williams et al. 2001). Such global changes have consequences for the functioning of terrestrial ecosystems,

including soil respiration, and a greater understanding of their interactive effects, is required to accurately estimate uncertainties in global climate change projections and predict ecosystem feedbacks to atmospheric CO<sub>2</sub> levels (Rustad 2008).

#### *1.4.1 Increased atmospheric CO<sub>2</sub>*

Rising atmospheric [CO<sub>2</sub>] generally increases rates of photosynthesis and consequently net primary productivity (Norby et al. 1999). The average increase in plant growth for a number of studies was 51% for grassy species and 42% for woody species (Zak et al. 2000). Numerous CO<sub>2</sub> experiments also report a general enhancement of belowground C cycling and increased rates of soil respiration (Bernhardt et al. 2006, Deng et al. 2010, Janssens et al. 1998, Lin et al. 2001, Pataki et al. 2003, Pregitzer et al. 2006, Trueman and Gonzalez-Meler 2005, Wan et al. 2007). Thus, although more C may be sequestered in plant biomass under elevated CO<sub>2</sub> than under ambient CO<sub>2</sub>, there may also be a simultaneous and greater output of either ‘newly added’ or ‘old’ C through respiration (Heath et al. 2005, Hungate et al. 1997, Trueman and Gonzalez-Meler 2005) and this is likely to limit the potential for increased C sequestration in terrestrial ecosystems exposed to increased atmospheric [CO<sub>2</sub>]. A meta-analysis by Jastrow et al. (2005) indicated that increased atmospheric [CO<sub>2</sub>] could result in potential additional storage of C (~5.6%) in soils due to generation and protection of root-derived C into micro-aggregates.

Fine root biomass, the total number of roots, and root length generally increase with atmospheric [CO<sub>2</sub>] (Iversen et al. 2008, Matamala and Schlesinger 2000, Pritchard et al. 2008, Rogers et al. 1994, Tingey et al. 2000), but specific root respiration

(respiration per unit root growth) is not always so affected (Matamala and Schlesinger 2000) or is not affected to such an extent that root and rhizosphere respiration are increased (Edwards and Norby 1999). This suggests that root and rhizosphere respiration may be more closely related to photosynthetic activity and belowground carbon allocation under rising atmospheric [CO<sub>2</sub>], rather than fine root growth *per se*.

Increased inputs of aboveground litter as well as increased root production, turnover and mortality under increased atmospheric [CO<sub>2</sub>] contribute to increased CO<sub>2</sub> efflux from soils by increasing the amounts of organic substrate available for heterotrophs (Deng et al. 2010, Zak et al. 2000). Heath et al. (2005) showed that increasing soil heterotrophic respiration with atmospheric [CO<sub>2</sub>] could be attributed mainly to enhanced production and turnover of root exudates. Furthermore, Trueman and Gonzalez-Meler (2005) found that the ‘priming’ effects of increased supply of root exudates enhanced decomposition of ‘old’ soil C. In contrast, litter produced by plants grown with increased atmospheric [CO<sub>2</sub>] may be richer in lignin and poorer in N than plants grown at ambient [CO<sub>2</sub>] (Henry et al. 2005, Knops et al. 2007), and this may adversely affect soil heterotrophic respiration. There is some evidence that concentrations of N in foliage in deciduous forest decline over time under increased atmospheric [CO<sub>2</sub>] relative to ambient atmospheric [CO<sub>2</sub>] (Norby et al. 2009). However, in other studies specific rates of litter decomposition were unchanged by CO<sub>2</sub> treatment (Henry et al. 2005, Knops et al. 2007). Increased atmospheric [CO<sub>2</sub>] may also have an indirect effect on organic matter decomposition through a shift in plant community composition. For example, there were more dicots, particularly legumes, in a grassland plot exposed to increased atmospheric [CO<sub>2</sub>] than in plots

under ambient conditions; this could enhance soil N availability and consequently alleviate N limitations to litter decomposition (Allard et al. 2004).

In water-limited systems, CO<sub>2</sub>-induced reductions in leaf stomatal conductance and plant transpiration (Deng et al. 2010, Field et al. 1995) can help increase availability of water in soil and thus soil respiration directly, as well as indirectly via enhanced photosynthesis, total belowground C input and substrate accessibility (Field et al. 1995, Pendall et al. 2003). The reverse may also be true in already-wet ecosystems - CO<sub>2</sub>-induced reductions in stomatal conductance may decrease instantaneous soil respiration rates due to increased diffusional constraints on soil-atmosphere gas exchange (Bader and Körner 2010). Clearly, many of the potential CO<sub>2</sub> effects depend on phenology (e.g. greater effects in developing forest stands than mature stands), the length of exposure to elevated CO<sub>2</sub> (e.g. less stimulation after long-term exposure) and soil conditions (Deng et al. 2010; Bader and Körner 2010). As an example, Tingey et al. (2006) found that when a covariance model was used to remove the influence of temperature, soil moisture and elapsed time from planting, increased atmospheric [CO<sub>2</sub>] had a significant negative effect on mean soil respiration over a 3-year period of growth of ponderosa pine seedlings. An overall decline in response of soil respiration over time may be attributed, at least in part, to declining rates of N mineralisation under conditions of increased atmospheric [CO<sub>2</sub>] (Bernhardt et al. 2006).

#### *1.4.2 Climate warming*



Climate warming can potentially convert terrestrial ecosystems from C sinks to C sources. This prediction is supported by a range of studies that show soil warming increases soil respiration by 20% overall, with forest ecosystems being more responsive to treatment than other ecosystems (Luo and Zhou 2006, Rustad et al. 2001). In a recent warming experiment, where soil temperature was increased by 4 °C above ambient, Schindlbacher et al. (2009) noted continuous increases in soil respiration by up to ~ 45-47% for 2 years, with nearly similar responses of autotrophic and heterotrophic components. Similarly, in a soil-only warming experiment, Bronson et al. (2008) recorded a 24% increase in soil respiration in the first year and an 11% increase in the second, following a 5 °C increase in soil temperature relative to the control treatment. Warming-induced prolongation of plant growing seasons and enhanced photosynthesis, may contribute to increases in soil respiration and increased N availability via organic matter mineralisation (Niu and Wan 2008, Wan et al. 2005). Warming may also increase soil respiration by enhancing mycorrhizal fungal biomass and throughput of recent photosynthate through roots and mycorrhizal fungi (Hawkes et al. 2008). However, several studies suggest initial responses of soil respiration to warming may dissipate with time as a result of any of the following: acclimation of respiring organisms including roots and microbes (Atkin et al. 2000, Bradford et al. 2008, Luo et al. 2001, Melillo et al. 2002); rapid depletion of readily available soil carbon pool (Hartley et al. 2007); decreases in fine root biomass (Bronson et al. 2008); reductions in soil moisture content (Rustad and Fernandez 1998).

Microbial acclimation to climate warming can include a shift in microbial community composition with increasing abundance of temperature-tolerant and

substrate-efficient fungi and possible reductions in the relative abundance or activity of bacteria (Zhang et al. 2005). Other researchers have discounted such acclimation as a major factor in soil respiration following climate warming (Eliasson et al. 2005, Hartley et al. 2008, Kirschbaum 2004), focusing instead on warming-induced limitation of substrate supply and low-carbon soils being especially susceptible, especially those from colder regions (Luo et al. 2001). Some soil warming experiments (e.g. mature *Picea abies* forests, Comstedt et al. 2006; grasslands, Luo et al. 2001, Wan et al. 2007) have shown no effects of increased soil temperature on soil respiration, while others have shown *reduced* respiration (by as much as 23-31%). Reductions in rates of respiration were attributed to complex interactions between physiology and substrate supply (Bronson et al. 2008). Clearly much more needs to be done to understand the effects of climate warming on soil respiration.

#### *1.4.3 Precipitation extremes*

Predicted interactions of soil respiration with future patterns of precipitation at regional scales (Almagro et al. 2009, Asensio et al. 2007, Boriken et al. 2006, Davidson et al. 2008, Harper et al. 2005, Jarvis et al. 2007, Stape et al. 2008) mostly emphasize precipitation extremes (high rainfall to prolonged droughts). In general, soil respiration rapidly increases following precipitation on soils previously submitted to a prolonged drought - a phenomenon known as the “Birch effect” (named after H.F. Birch, one of the first to describe this phenomenon, Davidson et al. 2008, Jarvis et al. 2007). Four reasons are commonly listed to explain this effect: (a) successive drying and wetting of soils disrupt soil aggregates and make the labile organic matter available for microbial decomposition; (b) dry spells kill soil

microorganisms that are subsequently decomposed when soils re-wet; (c) wetting of dry soils triggers a sudden growth and turnover of microbial biomass causing enhanced mineralisation of intercellular compounds in response to increased soil water potential; (d) microbes respond to osmotic shock during rewetting by releasing labile carbon-rich solutes that accumulated in their cytoplasm during the dry periods (Jarvis et al. 2007). Furthermore, displacement of CO<sub>2</sub> in soil pores following infiltration of rainwater may also contribute to rapid increases in soil CO<sub>2</sub> efflux i.e. usually called soil respiration (Huxman et al. 2004).

The Birch effect is more likely observed in ecosystems that experience longer droughts followed by heavy precipitation events (e.g. xeric ecosystems). Pulses of soil respiration accompanying rewetting following an extended dry period, increased annual emissions of CO<sub>2</sub> in Mediterranean ecosystems (Almagro et al. 2009, Jarvis et al. 2007) and the time taken for soil respiration to return to pre-precipitation, basal rates can range up to 30 days (Jarvis et al. 2007). The observation that a ‘rewetting index’ (precipitation over time elapsed between a precipitation event and soil respiration measurement), rather than soil water content, best described CO<sub>2</sub> efflux during summer drought emphasizes the need to consider precipitation rates and patterns in any assessment of overall carbon balance, especially in xeric ecosystems (Almagro et al. 2009).

Here too, however, there are examples of no effect of precipitation pulses on soil respiration (Muhr et al. 2008), or examples of short lived pulses producing a net decrease in soil respiration over the growing season (Harper et al. 2005). These

inconsistent results have been attributed to the overriding effects of substrate availability, e.g. as observed in a cold desert ecosystem in USA (Fernandez et al. 2006), or extreme water stress for plants or microbes, thereby limiting the response of soil respiration to precipitation events (Harper et al. 2005, Sponseller 2007). Furthermore, soil hydrophobicity can potentially increase with drought; this may delay the movement of water through the soil and thereby the response of soil respiration to precipitation (Muhr et al. 2008). Spatial variation in soil respiration is observed in ecosystems with irregular soil hydrophobicity or patchy vegetation cover. Water from precipitation can accumulate in places while in other places it may run off or be intercepted by plants, thus leading to uneven distribution of soil moisture (Muhr et al. 2008, Sponseller 2007). Wetting can also have a negative impact on aerobic respiration when soils become saturated, severely limiting gas exchange, and sometimes leading to an increase in soil CO<sub>2</sub> concentration but no immediate release of soil-respired CO<sub>2</sub> to the atmosphere (Smart and Penuelas 2005).

Extreme precipitation events, especially strong reductions in total precipitation, may also adversely affect autotrophic respiration simply by limiting net primary productivity (Harper et al. 2005). Heinemeyer et al. (2007) found a strong positive response of extraradical ectomycorrhizal respiration to changes in soil moisture as a result of prolonged drought followed rainfall. Absolute rooting depth is strongly and positively related to mean annual precipitation in many vegetation types (Schenk and Jackson 2002) and any increase in new plant C input to deeper soil layers may prime old soil carbon decomposition (Heimann and Reichstein 2008).

#### *1.4.5 Fire*

Effects of fire on soil CO<sub>2</sub> effluxes depend on the nature of the ecosystem, age of the forest stand, species composition, severity of the burn and frequency of fire. Immediately after a fire, soil respiration rates may be slowed due to the death of above ground vegetation, death of part of the soil microbial population (Hamman et al. 2007) or the consumption of substrate such as litter (Dumas et al. 2007). In the longer term, fire frequency and intensity will influence soil respiration by facilitating the rejuvenation of forests. Indeed, total soil respiration increases with the age of the stand (Gough et al. 2007) although it can be reduced again in the oldest stands (Czimczik et al. 2006).

The relative contribution of the heterotrophic or autotrophic component of soil respiration to total respiration following fire varies with time. In boreal forests, in the months and up to three years following fire, the decomposition of fire residues can create a peak in soil respiration that is of heterotrophic origins (Bond-Lamberty et al. 2004, Schulze et al. 2000). The part of heterotrophic respiration (Rh) is then reduced in younger stands -ie 5 to 70 year old (Bond-Lamberty et al. 2004, Czimczik et al. 2006, Gough et al. 2007). However, in warmer climates, Rh is not always affected by fire and can thus stay constant in the first years following the burn (Irvine et al. 2007, Meigs et al. 2009). Besides the increase or decrease of substrate available to microbes, Rh may be directly affected by changes in decomposer communities (Dumas et al. 2007) and in their structure especially immediately after fire (Barcenas-Moreno and Baath 2009, Hamman et al. 2007, Kara and Bolat 2009). Owing to a higher sensitivity to disturbance and reliance on plant and tree hosts,

fungi death is relatively greater than bacteria death as shown by the higher bacteria/fungi ratio in burnt compare to unburnt sites (Hamman et al. 2007, Kara and Bolat 2009). However total microbial biomass often returns quickly to its pre-fire level (Hamman et al. 2007, Kara and Bolat 2009) because the slow recovery of fungal growth is balanced with the ability of bacterial growth to recover quickly after fire and to a larger level than pre-fire conditions (Barcenas-Moreno and Baath 2009).

Indirectly, burning of the soil cover and understories can impact soil processes as the soil insulating (eg by moss and litter) and shading layers (eg shrubs, tree canopy) are greatly reduced up to the point of disappearing (Czimczik et al. 2006, Dumas et al. 2007). This results in sudden changes in soil temperatures due to the loss of the dampening effect provided by the insulating layer of vegetation whereas an overall increase and more extreme soil temperatures can be expected because of the greater exposition of soils (Czimczik et al. 2006, Dumas et al. 2007). The return of pre-fire soil temperatures in burnt sites is dependent on the recovery of the different strata of vegetation and can take less than two years (O'donnell et al. 2009). Soil moisture is also affected by the changes in vegetation cover since higher soil temperatures should increase direct evaporation whereas the possibly non-functioning understory and tree canopy would have transpiration greatly reduced (Dumas et al. 2007, Hamman et al. 2007). Because of the many influences of fire in forest ecosystems, the time lag between a fire event and the return to pre-fire level of heterotrophic respiration changes with intensity of fire and the composition and age of the stand.

A quick recovery of root respiration and therefore autotrophic respiration, possibly due to the densification of the understorey that benefited from the opening in the canopy after fire, can counterbalance the decrease in Rh and see a return of total soil respiration to pre-fire level within 2 years (Irvine et al. 2007). The consumption of the litter layer and opening of the canopy by more intense fires can also lead to an enrichment in species diversity (Dumas et al. 2007) and/or a shift in species abundance (Meigs et al. 2009). This in turn may be accompanied by a change in NPP that can partly compensate for the loss of aboveground live carbon and thus maintain the rates of soil respiration (Meigs et al. 2009).

Fire can greatly increase, decrease or not alter soil water repellency depending on fire intensity and behaviour and soil characteristics (ie texture, water content, type and quantity of SOM). In the case of mid intensity fires (ie soil temperature between 175 to 200°C), the moving underground of the repellent layer may lead to waterlogged soils whereas soil erosion might be favoured through the disappearance of the surface repellency. In contrast, high intensity fires with soil temperatures reaching between 250 and 300 °C tend to burn the repellent layer, thus decreasing soil hydrophobicity (Debano 2000). In any case, the change in soil water content resulting from the variation in hydrophobicity can delay the penetration and movement of water through the soil (Muhr et al. 2008) and therefore, affect decomposition rates (Davidson and Janssens 2006) or limit NPP (Harper et al. 2005) and as a consequence soil respiration can be impacted.

Nutrient availability in soils is immediately affected by fire to a degree that is dependent on the intensity of the fire, the soil physical properties and the moisture content of soils. Burning of litter and aboveground vegetation leads to loss of nutrients such as C, H, O, N, S and P through volatilisation during fire or surface run-off and wind dispersion of the ashes post fire. (Burke et al, 2005, Lagerstroem et al, 2009, Raison, 1979). Part of these nutrients may be transported to nearby unburnt ecosystems and may therefore impact on their soil respiration (Raison 1979). Therefore fire may not only impact burnt areas but also nearby systems. Less severe fires may free nutrients from the partially burnt aboveground vegetation, especially in forests where fuel is more abundant and incomplete burn are more common (Raison 1979). A return to pre-fire chemical condition usually takes approximately one year but may be as long as 30 years in the case of the more destructive fires (Khanna and Raison 1986, Raison 1979).

Intense fires reduce total N in soil but available forms of N are usually more abundant (Kutiel and Naveh 1987, Raison 1979). Fire may also stimulate N fixation or ammonification and therefore be followed by an increase in NH<sub>4</sub>-N (Khanna and Raison 1986, Raison 1979). Moreover, fire may stimulate the emergence of N-fixing species such as *Acacia sp* in Australia that will lead to enrichment in soil N (Raison 1979).

### ***1.5 Soil respiration in a changing world - interactions***

Terrestrial ecosystems are expected to experience multiple, concurrent and interacting, changes in global climate and these global changes will directly or



indirectly influence activities of roots and microbes in soil, and consequently soil respiration.

There are still very few studies e.g. (Deng et al. 2010, Norby and Luo 2004, Wan et al. 2007, Zhou et al. 2006) that have included multi-factor analysis of soil respiration (Rustad 2008). The available information shows that responses of soil respiration to multiple factors can be non-interactive or interactive and usually non-additive (Luo et al. 2008, Rustad 2008). For example, interactive effects of multiple global change factors were non-significant for soil respiration between warming and increased precipitation (Zhou et al. 2006), elevated CO<sub>2</sub> and warming (Edwards and Norby 1999, Niinisto et al. 2004), elevated CO<sub>2</sub> and nitrogen addition (Butnor et al. 2003), and increased atmospheric [CO<sub>2</sub>], air warming and water availability (Garten et al. 2009, Wan et al. 2007). On the other hand, Deng et al. (2010) found a strong interactive effect of increased atmospheric [CO<sub>2</sub>] and added nitrogen on soil respiration in young, subtropical forests and the combined effect of this two-factor treatment on soil respiration was greater (increased by 50%) than the either of the treatments alone (29% increase by elevated CO<sub>2</sub> and 8% increase by nitrogen addition). Similarly, belowground C turnover was affected more by warming plus increased atmospheric [CO<sub>2</sub>] than by increased atmospheric [CO<sub>2</sub>] alone, and the interaction was strongly mediated by nitrogen supply (Loiseau and Soussana 1999).

The highly variable, non-additive and complex responses of soil respiration to combinations of change factors have constrained our ability to predict feedback effects to future global scenarios. This underlines the need for further multi-factor

experiments in a range of ecosystems and over longer time-scales than used to date (Rustad 2008).

### ***1.6 Objectives***

The large annual emissions of CO<sub>2</sub> through soil respiration,  $98 \pm 12$  Pg CO<sub>2</sub>-C yr<sup>-1</sup>, signify the importance of soil respiration in the global C cycle. However, there is a lack of consensus and inconsistencies amongst studies on the role of the regulation of soil respiration by multiple abiotic (environmental) and biotic (plant-related) factors and the quantification of the soil respiratory components. This is mainly due to variations in the type and age of ecosystem studied, the timescale of measured responses, artefacts induced by methodologies employed (especially when seeking to partition soil respiratory components) or to the size of experimental units (e.g. glasshouse, whole-plant chambers, ecosystem-scale as in Free-Air Carbon Dioxide Enrichment experiments), and lack of consideration of the confounding influences of other factors not included in the experimental design. The global change drivers (such as elevated CO<sub>2</sub> concentration, elevated temperatures, precipitation extremes, rising N deposition) variably influence soil respiration with potential to further alter the global C cycle through feedback effects. The quantifying of soil respiration components and the understanding of the factors impacting on soil respiration is fundamental to predicting responses of soil respiration to global change and to improving predictions of future C sequestration in terrestrial ecosystems. (Luo et al. 2008, Rustad 2008).

In order to answer some of the above, this thesis focuses on understanding the effect of the predicted enriched atmospheric [CO<sub>2</sub>], the resulting changes in climate and their regional consequences on the belowground respiration of soil supporting native vegetation from south eastern Australia.

The following chapter presents the results of a study on the effect of elevated atmospheric [CO<sub>2</sub>] and changes in rainfall patterns on soil respiration. This study includes analysis of the components of soil respiration.

Finally, Chapter 3 describes a pulse labelling experiment using <sup>13</sup>CO<sub>2</sub> to trace the fate of carbon through trees submitted to a drought treatment replicating the changed rainfall pattern expected within the next fifty years. The comparison with carbon circulation through irrigated trees helps decipher the impact of rainfall patterns on carbon allocation belowground.

Chapters 4 to 5 examined the impact of native vegetation fires, also called bushfires, on soil respiration. First of all, I investigated the recovery of soil respiration post-fire in three different forest types (Chapter 4). Then, using the well known approach of trenching, coupled to the novel laboratory respirometer, I focused on the role of roots as both a direct and indirect contributor to soil respiration (Chapter 5). This chapter explores root priming effect and leads to the next (Chapter 6) where I aimed to examine the effects of litter input on soil respiration and the associated priming effect that may derive from it.

For such a large land mass, soil respiration in the Australian environment is little known. Most soil respiration studies have taken place in the northern hemisphere in conditions rarely encountered in Australia. This thesis will significantly improve the knowledge of soil respiration on the continent as well as elucidate some of the drivers of soil respiration at the global scale.

## ***Chapter 2***

### ***Effects of availability of soil water and atmospheric CO<sub>2</sub> on soil respiration in a *Eucalyptus saligna* plantation***

#### ***2.1 Introduction***

The atmospheric concentration of carbon dioxide (CO<sub>2</sub>) has increased by 36% since the mid 18<sup>th</sup> century, from a pre-industrial value of about 280 ppm to 379 ppm in 2005, and continues to increase by an average of 1.9 ppm/year (Forster et al. 2007). Increasing atmospheric [CO<sub>2</sub>] as well as increasing concentrations of other greenhouse gases in the Earth's troposphere, are likely contributing to global warming (Forster et al. 2007) and possibly to changes in patterns of precipitation whereby the frequency of intense rainfall events might increase by 5% - 10% per °C of warming (Allison et al. 2009). Belowground or soil respiration, is the major flux of CO<sub>2</sub> to the atmosphere. Averaged over 18 European forests, soil respiration contributed 69% of the total terrestrial ecosystem respiration (Janssens et al. 2001). In other words, an estimated 10% of the CO<sub>2</sub> of the atmosphere cycles through soils each year (Raich and Potter 1995), which represents about ten times the amount of CO<sub>2</sub> produced by burning fossil fuels.

Despite its importance in the carbon cycle, soil respiration is not as well known as its above-ground counterpart. In part this is due to the difficulty of isolating and quantifying soil respiration components between autotrophic and heterotrophic sources. Autotrophic respiration can be clearly identified as root respiration. Because

of methodological constraints, respiration from rhizosphere microorganisms, including mycorrhizal fungi, is usually coupled with the autotrophic respiratory component and the argument for this is that these microorganisms are highly dependent on plants for their supply of recently-fixed carbohydrates via photosynthesis. In a strict sense, soil respiration from these sources should however be classified as heterotrophic respiration. Heterotrophic respiration also includes respiration from free-living microorganisms in soil (Cisneros-Dozal et al. 2006, Hogberg et al. 2001). While root respiration has been evaluated as representing 10 to 90 % of total soil respiration in different studies (Hanson et al. 2000), the development of new techniques has allowed to refine these values to an average of over 50 % in a bracket of 24% to 81% (see Table 1.1, Chapter 1).

Soil temperature is usually regarded as the most important regulator of soil respiration (Fang and Moncrieff 2001) by either facilitating litter and organic matter decomposition (Schindlbacher et al. 2009) or impacting directly on rhizosphere respiration. In recent years, however, soil moisture has risen in importance as a result of increased study. Soil moisture can become the main driver of soil respiration at warmer temperatures (e.g. > 16 °C, Almagro et al. 2009). Respiration in many soils is also highly moisture-dependent at lower soil moisture contents (< 20%, Almagro et al. 2009, Jassal et al. 2008) where it is frequently difficult to disentangle direct moisture effects from its indirect effects on, for example, substrate and enzyme mobility and nutrient availability (Harper et al. 2005).

Increased atmospheric [CO<sub>2</sub>] often, but not always, increases rates of photosynthesis and net primary productivity (Norby et al. 1999). Photosynthesis is, in turn, a major regulator of autotrophic respiration (Bhupinderpal-Singh et al. 2003, Ekblad et al. 2005, Hogberg et al. 2008, Hogberg et al. 2001, Kuzyakov and Cheng 2001, Wan and Luo 2003) and, globally, soil respiration is positively related to aboveground net primary production (Raich and Schlesinger 1992).

In addition, increased atmospheric [CO<sub>2</sub>] can lead to reductions in leaf stomatal conductance and plant transpiration, helping conserve soil water. The possibility of indirect promotion of soil respiration via this process has some support (Deng et al. 2010, Field et al. 1995, Pendall et al. 2003). On the other hand, increases in soil water content in wet ecosystems due to reductions in stomatal conductance could increase impairment of gas diffusion, particularly between soil and atmosphere (Bader and Korner 2010). To varying degrees, increases in atmospheric [CO<sub>2</sub>] and resulting changes in climate are thus expected to affect most components of soil respiration.

Because of their size, trees and forests are difficult subjects for field-based studies of effects of changing atmospheric [CO<sub>2</sub>] and water availability that usually require costly infrastructure. That infrastructure has included open top chambers (OTC, Drake et al. 1989, Leadley and Drake 1993, Mandl et al. 1973, Norris et al. 1996) and closed top or whole tree chambers (WTC, Barton et al. 2010, Kellomaki et al. 2000, Medhurst et al. 2006) for single trees, and Free Air CO<sub>2</sub> Enrichment (FACE, Hendrey et al. 1999, Lewin et al. 1994, Nagy et al. 1994) for forest stands.

Here I present the results of a study of soil respiration using Whole Tree Chambers to grow *Eucalyptus saligna* over a period of 12 months. *E. saligna* is native to Australia but used around the world as a fast-growing hardwood suited to plantations. The aim of this study was to quantify the effects of atmospheric [CO<sub>2</sub>] and water availability on soil respiration with the straightforward hypothesis that both increased atmospheric [CO<sub>2</sub>] and increased water availability would increase rates of respiration.

## ***2.2 Materials and methods***

### ***2.2.1 Site description***

The Hawkesbury Forest Experiment (HFE) is located at the University of Western Sydney campus in Richmond, North-West of Sydney, Australia (Lat. 33°36'40" S, Lon. 150°44'26.5" E). It sits on the alluvial floodplain of the Hawkesbury River at elevation 25 m a.s.l. The soil is a chromosol, sandy in texture, with a pH around 5.2 in the first 20 cm, C:N ratio around 20.6 and is low in C 1.1% within first 20 cm), N (0.07% in first 20 cm) and P (8 ppm within first 20 cm).

Average annual temperature in the area is 17 °C, varying between a mean minimum temperature for the coldest month of 3°C and a mean maximum temperature for the hottest month of 29°C. The mean annual precipitation is 801 mm spread over an average 74.6 days receiving over 1mm of rainfall mostly in the spring months of November and summer month of February but with a strong inter annual variation.



One-month old Sydney blue gums (*Eucalyptus saligna*) were planted in May 17<sup>th</sup> and 18<sup>th</sup> 2007, 2.6 metres apart along rows separated by gaps of 3.85 metres. Twelve whole tree-chambers (WTC) were installed, each around one tree. The WTC are well described in Barton et al (2010) and Medhurst et al (2006). Briefly, the WTC consist of an alloy frame covered initially with polyvinyl chloride (PVC) panels. The PVC was later replaced with ethylene-tetrafluoroethylene (ETFE) that offers better UV and light transmittance. The WTC are 3.25 m in diameter and 2.5 m high, in the shape of a slightly tapered cylinder surmounted by a three meter tall cone. The cylindrical part was extended to 5.15 m in September 2008 to allow for the rapid growth of the trees. A PVC floor situated 0.45 m above ground and sealed around the stem of the tree, separates the soil compartment from the above ground compartment. The chamber walls are buried into the soil just outside of a root barrier that extend to a depth of 1 m. The underfloor compartment is constantly ventilated but can also be sealed when needed.

Two environmental variables could be controlled inside the chambers and comprise the independent variables for this experiment: soil moisture and atmospheric [CO<sub>2</sub>].

At the start of the experiment, to ensure a good establishment of the trees, all WTC were irrigated by sprinklers with 10 mm of water every third day. A first drought cycle started on 16 February 2008 when all irrigation ceased for half of the experimental plots. The imposed drought was broken on 18 September 2008 after

more than 200 days. A second drought cycle started on 26 October 2008 and lasted until 4 March 2009 (a further 129 days).

Atmospheric [CO<sub>2</sub>] was either ambient or elevated and the treatment started at the day of planting. Ambient [CO<sub>2</sub>] in the ambient chambers matched that of the external environment of the site and varied between 380 ppm and 500 ppm, mainly due to a typical diurnal cycle (higher at night). Elevated [CO<sub>2</sub>] was maintained 240 ppm above ambient atmospheric [CO<sub>2</sub>] (Barton et al. 2010).

There were three replicates of each possible combination of water and CO<sub>2</sub> treatments:

- ambient [CO<sub>2</sub>]-drought,
- ambient [CO<sub>2</sub>]-irrigated,
- elevated [CO<sub>2</sub>]-drought,
- elevated [CO<sub>2</sub>]-irrigated.

Six additional control trees and treatments (three drought, three irrigated) were established to evaluate the WTC effect on dependent variables such as air temperatures or water vapour deficit. (Barton et al. 2010). A frame bearing a plastic sheet was installed 300 to 200 mm over the soil and around the stem of each tree, preventing rainfall from reaching the soil. Sprinklers controlled the moisture delivered to the soil around the trees in accordance with the irrigated and drought treatments inside the WTC (Barton et al. 2010).

Two mini-rhizotron tubes were also installed in each of the WTC and control plots at a distance of 500mm and 1000mm from the base of the trees. These were used to monitor the growth of roots to a depth of up to 600 mm.

### 2.2.2 *Belowground respiration measurements*

Static chambers were used to measure soil respiration. At first, four chambers were inserted in the soil in February 2008 within each WTC as well as in the control plots to a depth of about 50mm: Two chambers were placed at about 500 mm from the stem of the trees and two more at a distance of about 1000 mm.

In September 2008, the original four chambers were replaced with two sets of mesh-collar chambers based on the design by Heinemeyer et al. (2007). Each set consists of one 50 mm tall x 200 mm diameter sharpened collar inserted to 10 mm into the soil and maintained in place with pegs and two 250 mm tall x 200 mm diameter cores inserted to a depth of 200 mm into the soil. The cores were equipped with four 50 x 50 mm windows distributed evenly on their side 65 mm from the top edge of the core. Each window was fitted with a 38- $\mu$ m nylon mesh or a 1- $\mu$ m nylon mesh that respectively allows or prevents the in-growth of mycorrhizal hyphae inside the cores. A deeper 700 mm long and 100 mm diameter core without windows was also inserted in each WTC or control plot. It is designed to exclude any roots, including the deeper growing ones. Soil respiration was measured with a Vaisala carbocap GM 343 (Vaisala, Helsinki, Finland) mounted on a 5.25 liter lid. The lid-Vaisala-collar association works as a static chamber equipped with its own CO<sub>2</sub> analyser. The lid was left on the collars for ten minutes out of which only the last eight were

considered in order to calculate the rate of evolution of CO<sub>2</sub>. The recording during the first two minutes was discarded in order to allow the [CO<sub>2</sub>] to stabilise.

Measurements were taken on a monthly basis from March to May 2008, and again from September to December 2008 and then every three weeks until 9 February 2009. Soil respiration was measured a few hours before and after midday at the warmest time of the day. Two rounds of overnight measurements were made on one elevated WTC, one ambient WTC and one control plots for both irrigated and drought treatments. Results showed that there was no major diurnal variation in soil respiration and maximum rates were recorded at any time of day or night. At the time of measurements, soil temperature was recorded on each plot at 100 mm depth and soil volumetric water content at 120 mm depth.

During the March 2009 rewatering campaign of the droughted WTC, and due to a pulse labelling experiment that ran for the previous month, the whole under floor space of the WTC was used as a static chamber. The Vaisala GMP 343 was fitted through the sidewall of the under floor compartment of the WTC and a computer fan was used to mix air evenly. A first measurement was conducted on 3 March for basal data and then on five occasions starting 5 March 8am until 6 March 5pm. More measurements were taken once on each of the next three days and finally on 11 and 13 March. This regime of measurement permitted a detailed characterization of the response of soil respiration to rewatering in comparison with the brief analysis after the first rewatering in September 2008.

Root data were collected with the mini-rhizotron on 4 dates between 12 March and 26 June 2008.

Microbial biomass of the top 10 cm of the ambient WTC was analysed as part of the pulse labelling experiment (see Chapter 3). The fumigation extraction method described in Vance et al. (1987) was used. Dissolved organic carbon was extracted from fumigated and non-fumigated soils with 0.125M K<sub>2</sub>SO<sub>4</sub>.

### *2.2.3 Statistical analysis*

Data were analysed using two-way ANOVA with [CO<sub>2</sub>] treatments and irrigation treatments as factors for each time point. Linear regression was used to investigate the influence of soil temperature and soil moisture on soil respiration.

## **2.3 Results**

### *2.3.1 Soil respiration and aboveground biomass*

Rates of soil respiration presented a similar pattern to the rates of growth of the aboveground biomass regardless of the watering and [CO<sub>2</sub>] treatment (Figure 2.1). The ratio soil respiration:aboveground growth rate was greater for the earlier soil respiration measurements in September and October 08 (between 8 and 23). They then declined to values between 4.5 and 9. The irrigated-elevated [CO<sub>2</sub>] WTC presented the greatest ratio, starting at 23 and ending at 6.5 (Figure 2.1 d).

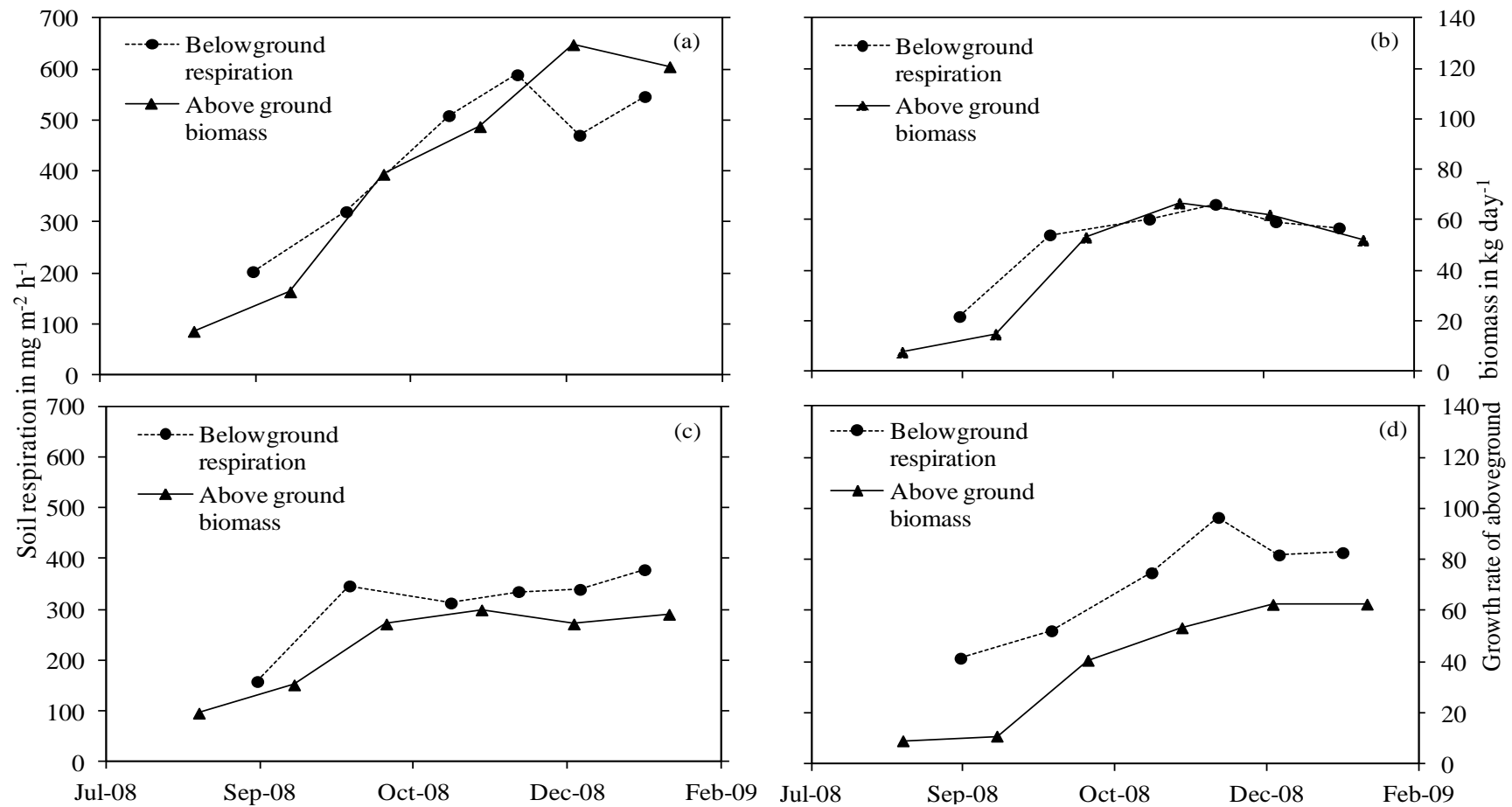


Figure 2.1.

Rates of belowground respiration and aboveground biomass of the *Eucalyptus saligna* at HFE in (a) Irrigated-ambient [CO<sub>2</sub>], (b) Droughted-elevated [CO<sub>2</sub>], (c) Droughted-ambient [CO<sub>2</sub>], (d) Irrigated-elevated [CO<sub>2</sub>] whole tree chambers (WTC). Results are averaged over three replicates of WTC. Aboveground biomass data provided by Craig Barton.

### 2.3.2 Irrigation treatment

On most occasions and independent of [CO<sub>2</sub>] treatment, rates of soil respiration were significantly faster when water was applied than when it was withheld (Figure 2.2, Table 2.1). There was one exception for the second measurement in April 2008 when rates were not significantly different between drought and irrigated treatments. Furthermore, in October 2008 and March 2009, there were sudden and short-lived increases in soil respiration after the drought treatment was stopped by irrigating the droughted plots. Rates of respiration were transiently faster for these previously dry soils than the irrigated soils, albeit the difference was not significant (Figure 2.2 and 2.3, Table 2.1, two-way ANOVA,  $P > 0.05$ ).

Table 2.1.

Results of two-way ANOVA analysis of the effect of the irrigation treatment, [CO<sub>2</sub>] treatment and their combination on soil respiration within WTC. WTC effect is the result of ANOVA analysis of soil respiration from ambient WTC and control plots. All results in bold are significant ( $P < 0.05$ ).

	Irrigation		CO <sub>2</sub> treatment		Irrigation x CO <sub>2</sub>		WTC effect	
	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)	Fvalue	Pr(>F)
11/03/08	9.7	<b>0.014</b>	2.8	0.131	0.6	0.454	46.0	<b>0.000</b>
3/04/08	0.0	0.916	0.0	0.894	0.0	0.938	0.0	0.907
15/05/08	9.1	<b>0.017</b>	0.8	0.392	0.5	0.490	0.7	0.434
8/09/08	4.7	0.062	0.5	0.504	0.7	0.434	5.7	<b>0.043</b>
8/10/08	0.1	0.710	2.1	0.182	0.0	0.864	0.1	0.806
10/11/08	6.8	<b>0.031</b>	2.0	0.193	1.4	0.266	7.3	<b>0.027</b>
2/12/08	25.9	<b>0.001</b>	1.9	0.200	1.6	0.237	17.7	<b>0.003</b>
22/12/08	9.8	<b>0.014</b>	1.8	0.215	0.1	0.826	9.6	<b>0.015</b>
12/01/09	10.8	<b>0.011</b>	6.3	<b>0.036</b>	0.2	0.682	6.0	<b>0.040</b>
9/02/09	7.1	<b>0.029</b>	1.4	0.268	0.1	0.810	11.0	<b>0.011</b>

The drought treatment had a greater effect on ambient [CO<sub>2</sub>] WTC and control plots than elevated [CO<sub>2</sub>] WTC. Droughted control plots showed significantly (two-way ANOVA,  $P < 0.05$ ) slower rate of soil respiration compared to irrigated plots in April

and from December to January inclusive, with a less marked difference in November ( $P=0.057$ ). Inside the ambient [CO<sub>2</sub>] WTC during the summer months, the rate of soil respiration was significantly ( $P<0.05$ ) faster in wet than droughted plots, except for the second set of measurements in December when differences were only marginally significant ( $P=0.067$ ). Elevated [CO<sub>2</sub>] WTC showed no significant difference in soil respiration in spring and autumn but significantly ( $P<0.05$ ) faster rates in irrigated than droughted chambers during other months.

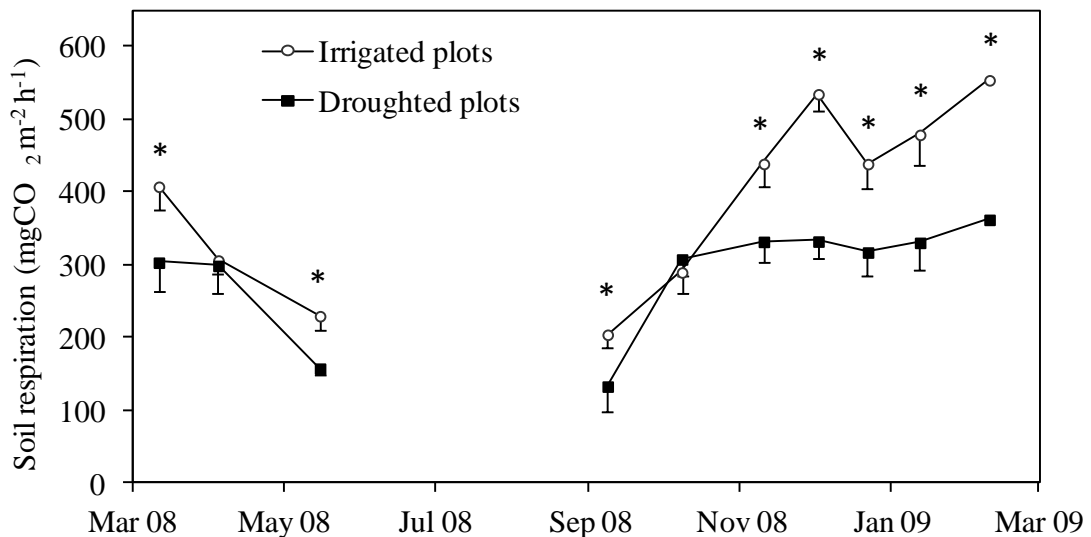


Figure 2.2.

Time series of soil respiration measured at the Hawkesbury Forest Experiment (HFE) between March 2008 and February 2008 plotted against the watering treatment. Each line is the mean of six replicates across three ambient WTC and three elevated [CO<sub>2</sub>] WTC. Stars show a significant difference ( $P<0.05$ ) in soil respiration calculated with two-way ANOVA.

### 2.3.3 Rewatering effect

In March 2009, after 129 days of drought, the droughted plots were irrigated. There was an immediate (within 24 hours of rewatering) switch from faster rates of soil respiration in irrigated plots to faster rates in droughted plots (see Figure 2.3). This effect lasted for the remainder of the measurements over the following eight days.



Soil respiration for all treatments in the WTC followed the same pattern during this time. After five days, soil respiration in the elevated [CO<sub>2</sub>]-droughted WTC began declining compared to other plots.

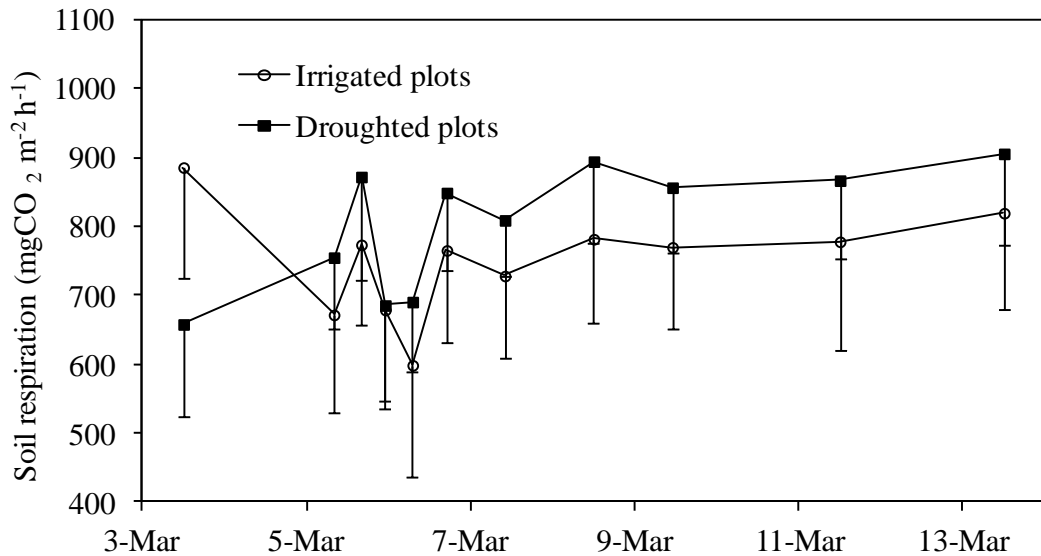


Figure 2.3.

Time series of soil respiration following rewatering on 4 March 2009. Soil respiration was measured using the under-floor space of the WTC as a static chamber. Mean of six replicates across three ambient WTC and three elevated WTC subjected to drought treatment (receiving no water) or irrigated treatment.

#### 2.3.4 Elevated [CO<sub>2</sub>] treatment

Rates of soil respiration were slightly greater in ambient than elevated WTC except in March and April 2008 (Figure 2.4). This difference was not significant (ANOVA,  $P > 0.05$ ), apart from January 2009.

Soil respiration followed a similar annual pattern in elevated and ambient [CO<sub>2</sub>] WTC. Rates in ambient [CO<sub>2</sub>] WTC ranged from a low 181 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> in September 2008 to a high 502 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> in December 2008. Elevated [CO<sub>2</sub>] WTC released a minimum of 158 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> in September 2008 and a maximum of 416 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> in December 2008.

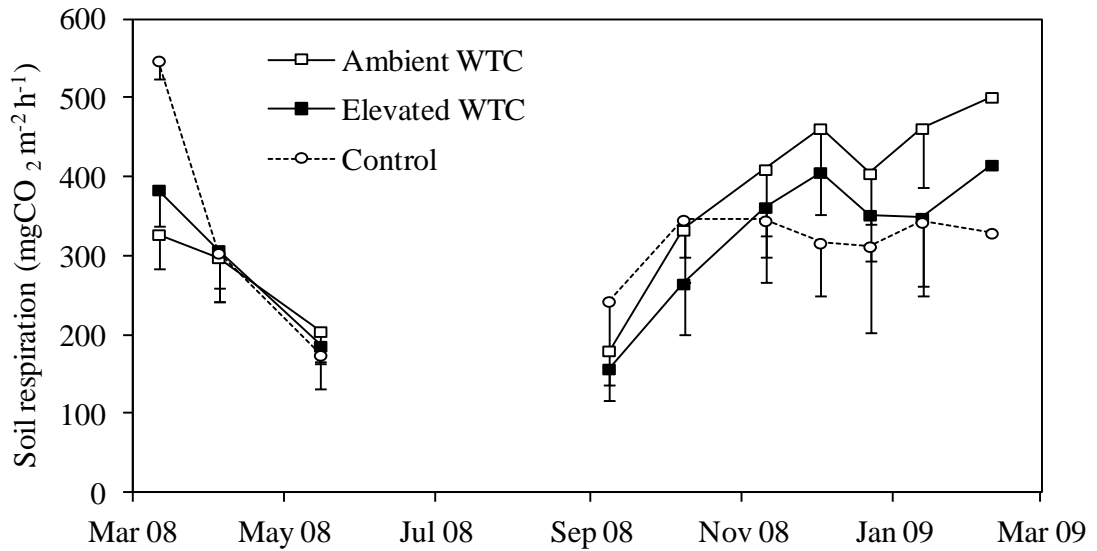


Figure 2.4.

Time series of soil respiration at HFE averaged over six control plots, six elevated [CO<sub>2</sub>] WTC and six ambient [CO<sub>2</sub>] WTC across irrigation and drought treatment. Standard error bars are displayed in the negative direction.

### 2.3.5 Interaction between treatments

No interaction between [CO<sub>2</sub>] and watering treatment was recorded for any measurement day or across all measurements.

### 2.3.6 Soil temperature and moisture

Regardless of treatment, rates were very variable throughout the year for WTC and control plots (Figure 2.4). They were greatest in the warmer summer months of March 2008 and February 2009 and least during the colder winter months of September and May 2008.

Soil moisture was a more prominent driver of soil respiration than soil temperature (Table 2.2). A significant link between soil temperature and soil respiration could

only be established on two occasions across all treatments. On the other hand, there was a significant effect of soil moisture on soil respiration during six out of the nine measurements between March 2008 and February 2009. Except in October 2009, following rewatering of droughted plots, soil temperature only had an impact on the soil respiration of irrigated trees for five out of the nine measurements between March 2008 and February 2009 (linear regression,  $P < 0.05$ ). Soil respiration of all [CO<sub>2</sub>] treatments, controls or irrigated treatments was affected at some times by soil moisture. In the contrary, soil temperature of droughted plots had no effect on soil respiration.

After the March 2009 rewatering, there was a small increase in microbial biomass of the top 10 cm of soil of the droughted-ambient [CO<sub>2</sub>] WTC for up to 13 days (Figure 2.5). However, the increase was not such that microbial biomass in the droughted WTC became greater than in the irrigated WTC.

Table 2.2.

*P* values per treatment and for each treatment from linear regression analysis between soil temperature and soil respiration and soil volumetric water content and soil respiration.

		3 Apr 08	15 May 08	8 Sep 08	8 Oct 08	11 Nov 08	3 Dec 08	23 Dec 08	13 Jan 09	9 Feb 09
Soil temperature	All	0.955	0.128	0.297	<b>0.000</b>	0.938	0.969	0.351	0.125	<b>0.024</b>
	Controls	0.197	0.925	0.989	<b>0.041</b>	0.841	0.612	0.148	0.194	0.240
	Ambient	0.272	0.334	0.602	<b>0.007</b>	0.255	0.067	0.697	0.674	0.282
	Elevated	0.988	0.720	0.266	<b>0.001</b>	0.437	0.910	0.741	0.706	0.222
	Droughted	0.402	0.492	0.126	<b>0.019</b>	0.204	0.070	0.327	0.863	0.990
	Irrigated	<b>0.047</b>	<b>0.033</b>	0.204	<b>0.007</b>	<b>0.023</b>	0.210	0.542	<b>0.010</b>	0.515
Soil moisture	All	<b>0.015</b>	<b>0.049</b>	0.134	<b>0.001</b>	<b>0.005</b>	0.157	0.502	<b>0.001</b>	<b>0.005</b>
	Controls	0.130	0.487	0.134	0.504	0.153	0.511	0.238	<b>0.035</b>	<b>0.002</b>
	Ambient	0.955	0.414	0.075	<b>0.007</b>	<b>0.004</b>	0.080	0.111	<b>0.016</b>	0.367
	Elevated	<b>0.005</b>	0.094	<b>0.013</b>	<b>0.008</b>	0.259	0.520	0.157	<b>0.042</b>	0.158
	Droughted	0.973	0.366	0.991	0.109	0.235	0.190	0.784	0.688	0.741
	Irrigated	0.247	0.958	0.484	<b>0.033</b>	0.326	0.411	0.918	<b>0.031</b>	0.755

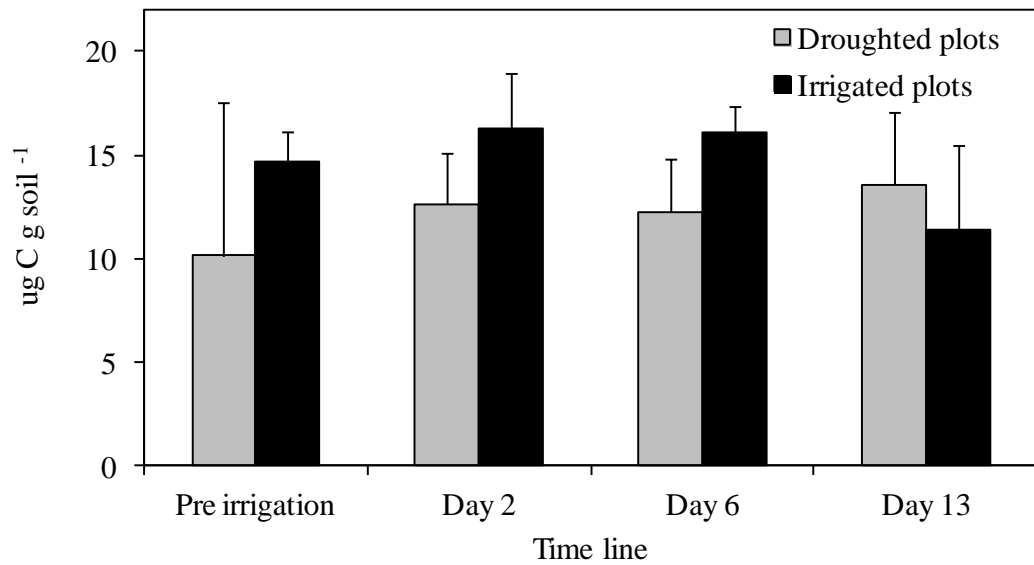


Figure 2.5.

Soil microbial biomass (0-10 cm depth) from the ambient WTC before and up to 13 days after the 5 March 2009 rewatering.

### 2.3.7 Partitioning of components of respiration and the 'WTC effect'

There was no detectable difference in rates of soil respiration measured from the mesh-collars compared with the rates measured from the short collars.

There was a significant difference in rates of soil respiration between open-air control and ambient [CO<sub>2</sub>] WTC in March, September and from November 2008 to February 2009. However in March and September 2008, respiration from the control plots was 68% and 35% greater than from the ambient [CO<sub>2</sub>] WTC whereas the trend was reversed in May 2008 and from November 2008 with 15 to 34% slower rates of soil respiration in control plots than WTC (see Figure 2.4). There were greater and more frequent differences within the drought treatment than within the irrigated treatment: out of the ten measurements, on seven occasions there was a greater difference between droughted control and droughted WTC than between irrigated

controls and irrigated WTC. Soil respiration from the droughted controls ranged from -55% to +82% of those measured in droughted WTC, whereas respiration in irrigated controls plots varied from -27% to +56% of those measured in respective irrigated WTC.

### 2.3.8 Root growth

The trees in droughted WTC regardless of [CO<sub>2</sub>] treatment, irrigated-elevated [CO<sub>2</sub>] WTC and irrigated controls grew more roots at depth between 0 and 30 cm than 30 to 60 cm (Figure 2.6). Trees in irrigated-ambient [CO<sub>2</sub>] WTC and droughted controls grew more roots at 30 to 60 cm depth.

A large amount of roots was present at depth below 30 cm. Most of these roots were thus below the maximum depth of the mesh-collar chambers (ie at depths >20 cm).

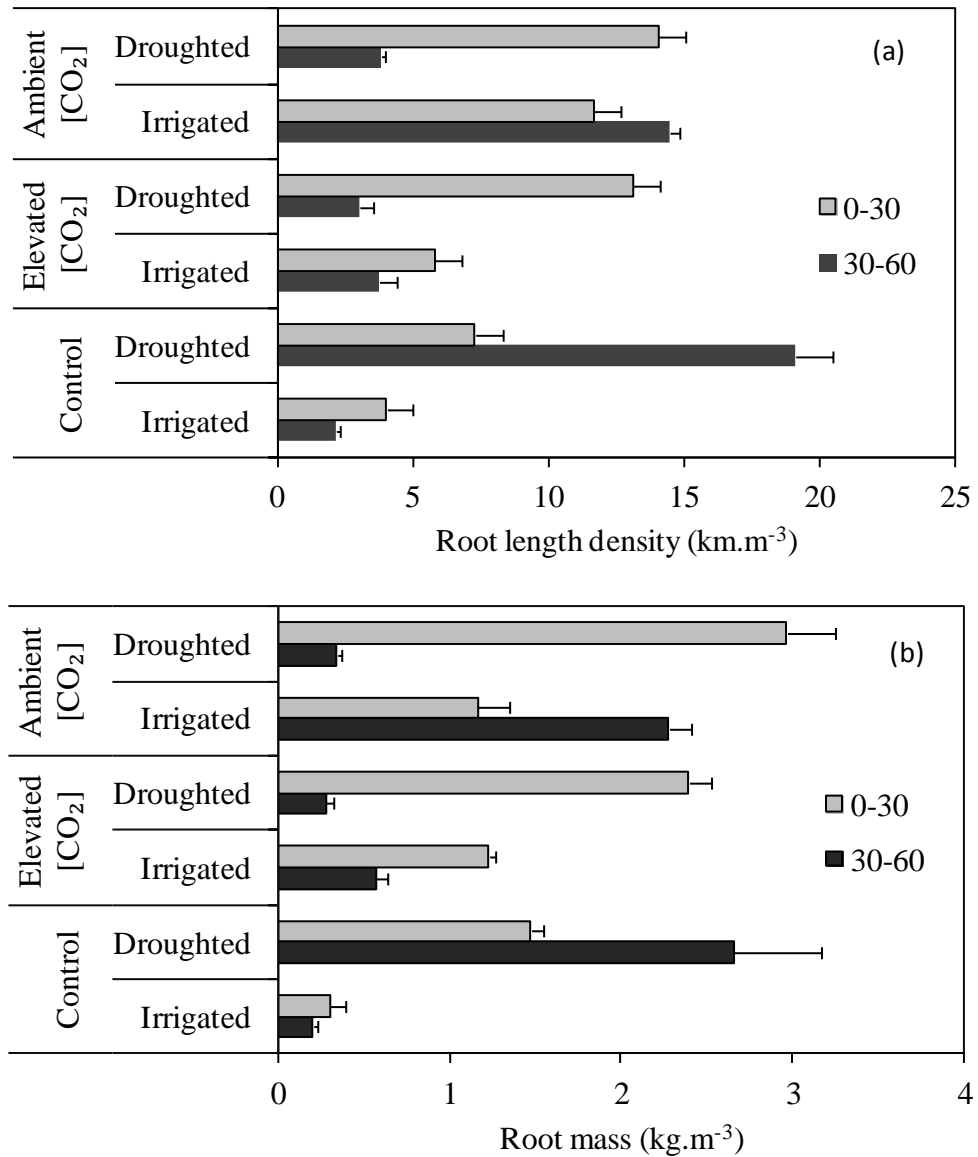


Figure 2.6.

Root length density (a) and root weight (b) recorded with a mini-rhizotron on four dates. Results are averaged over the four dates and reported as mean across three replicates for each [CO<sub>2</sub>]-irrigation treatment combination and over the three control for each irrigation treatment. Data provided by Tarryn Turnbull.

## 2.4 Discussion

### 2.4.1 Irrigation treatment

Greater soil respiration from watered WTC than from droughted WTC are consistent with results of many other studies. In Brazil, irrigated clones of *Eucalyptus grandis* x

*urophylla* produced a faster rate of soil respiration than their rain-fed counterparts over two years (Stape et al. 2008). Furthermore, the drought and rainy periods of the year were matched by respectively slow and fast rates of soil respiration in eucalypts forest (Stape et al. 2008) as well as in conifers, broadleaf mixed and evergreen broadleaf forests (Tang et al. 2006).

Only when the HFE experiment was established and when long drought cycles were broken, were rates of soil respiration in droughted plots faster than those in irrigated plots. The lack of significant differences in March and April 2008 can be attributed to the short period of treatment (i.e. after drought was established on the droughted plots). The elapsed time did not allow for clearer treatment effects. The switches in soil respiration in October 2008 and in March 2009 following irrigation of the droughted plots present a different scenario. The sudden increase in soil respiration after rewatering of soils previously submitted to a lengthy dry episode, is called “Birch effect” named after H.F. Birch, one of the first to describe this phenomenon (Jarvis et al. 2007). Four reasons are commonly listed to explain the Birch effect:

1. Successive drying and wetting of soils make organic matter available for decomposition by breaking soil aggregates.
2. The dry spells kill soil micro-organisms which are subsequently decomposed when wetting occurs, hence releasing nutrients.
3. Wetting triggers a sudden growth of microbial biomass and fungal hyphae.
4. The rewetting generates an osmotic shock to the microbes that respond by releasing carbon rich solutes that accumulated in their cytoplasm during the dry periods.



Microbial biomass increased after the March 2009 rewetting of the droughted plots at HFE but not significantly so. An increase in microbial activity rather than biomass is the most likely explanation for the peak in respiration. As the HFE was not submitted to many drying and wetting cycles and the sandy texture of the soil would not support many aggregate, the first of the four reasons for the Birch effect is probably not relevant to this site. This leaves decomposition of micro-organisms killed by the drought, and the consequent release of carbon (through osmotic shock) as the most likely explanation of the sudden increase in soil respiration) following the watering of the droughted plots at HFE.

Recent studies have shown both an increase in yearly forest soil CO<sub>2</sub> emissions (Jarvis et al. 2007) and no extra release of CO<sub>2</sub> (Muhr et al. 2008) following the wetting of previously dry soils. In the present study, the watering of droughted plots had no significant effect on soil respiration. Moreover, the observed increase in respiration rates of the late September 2008 rewatering observed in October 2008 had disappeared within one month. Similarly, in March 2009 rates of soil respiration in the three droughted-elevated [CO<sub>2</sub>] WTC began declining almost immediately after rewatering. These results suggest that over a year, no extra CO<sub>2</sub> would be emitted due to the Birch effect. The response of soil respiration to precipitation following a period of drought is sometimes proportional to the length of the drought and the amount of rain (Almagro et al. 2009). Results presented here reflect a particular set of circumstances that can form the basis of future work. As the Australian climate of the 21<sup>st</sup> century is predicted to bring longer dry spells broken

by heavier rainfall episodes (Csiro and Bom 2007), models to predict soil respiration in Australia will have to include models and observations of future weather patterns.

A majority of measurements between March 2008 and February 2009 presented a positive relationship between soil temperature and soil respiration on irrigated plots and between soil moisture and respiration across all treatments. Jassal et al. (2008) noted that water stress in a Douglas fir forest significantly reduced carbon dioxide emissions from soils. Furthermore, under a threshold of 11 m<sup>3</sup>.m<sup>-3</sup> of soil water at 4 cm depth, soil respiration was not affected by soil temperature, but was linearly dependent on soil moisture content.

Available evidence suggests trees within droughted plots accessed water from deep soil layers (Duursma et al. 2011). It seems likely that root respiration was maintained at normal rates by that supply of water.

#### *2.4.2 Elevated [CO<sub>2</sub>] effect*

Most studies note an increase in soil respiration when vegetation is exposed to an elevated [CO<sub>2</sub>] atmosphere (Bernhardt et al. 2006, Hamilton et al. 2002, Hoosbeek et al. 2007, Lin et al. 2001, Pajari 1995, Wan et al. 2007, Zak et al. 2000). However, in other cases including this study, there has been no effect (Bader and Korner 2010, Oberbauer et al. 1986) or even a negative effect (Tingey et al. 2006). The intensity of stimulation of soil respiration varies with the age or the composition of the stands (King et al. 2004, Pregitzer et al. 2006) and the length of exposition to elevated [CO<sub>2</sub>] (King et al. 2004). When observed, increases in respiration are greater in young stands and during initial years of fumigation. Genotype can also be a factor as

reported by Kasurinen et al. (2004). They observed a positive effect of elevated [CO<sub>2</sub>] on soil respiration under one clone of silver birch (*Betula pendula*) whereas the effect was negative under another clone with the difference increasing after three years. Most studies of the effect of elevated [CO<sub>2</sub>] on soil respiration under woody plants have been conducted in the northern hemisphere on local species. The HFE is one of the first climate change experiment that is field based and using *Eucalyptus* sp. While it could be hypothesised that the present experiment follows the bulk of the literature, the results suggest specific characteristics of *Eucalyptus saligna* (e.g. patterns of root growth) modify the climate response relative to other species.

Increases in soil respiration caused by exposure to atmosphere with an elevated [CO<sub>2</sub>] can be partly due to an increase in aboveground litter and root production and turnover leading to an increase in heterotrophic respiration (Matamala and Schlesinger 2000, Pritchard et al. 2008, Tingey et al. 2000). Here, the floor in the WTC prevented any aboveground litter reaching the soil. The input of microbial decomposers feeding on surface litter was thus missing.

Autotrophic respiration can be enhanced through an increase in fine root biomass, total number of roots and root length (Matamala and Schlesinger 2000, Pritchard et al. 2008, Tingey et al. 2000). However specific root respiration (respiration per unit of root biomass) is not always changed in elevated [CO<sub>2</sub>] experiments (Matamala and Schlesinger 2000, Tang et al. 2006) or is not affected to such an extent that roots and rhizosphere respiration increases (Edwards and Norby 1999).

### 2.4.3 Soil respiration partitioning

Respiration rates recorded here are at the upper limit of those in the literature but on par with rates observed in forest ecosystems (Subke et al. 2006). This was expected as trees belonging to *Eucalyptus* species are fast growing and recent photosynthates contribute strongly to root respiration (Hogberg et al. 2001). This point is confirmed by the closeness of the rates of above ground biomass growth and soil respiration during the measurement period. As a matter of comparison, the WTC at the HFE were previously used in Sweden for 40-year-old spruce trees (Medhurst 2006) whereas during the two years of fumigation at the HFE the *Eucalyptus saligna* grew from a seedling stage up to 10 metres tall.

Mesh-collars did not create any difference in soil respiration despite the roots inside being severed from the trees and collars with the 1- $\mu$ m mesh window preventing in-growth of mycorrhizal hyphae (Heinemeyer et al. 2007). In April 2009, after the trees were harvested, the mesh-collars were removed from the soil of the WTC. The integrity of the collars had not been compromised during the time they spent in the soil of the WTC and control plots. Washing and isolating of the roots from inside the mesh-collar chambers led to the observation that roots did not decompose and some kept growing after installation of the mesh-collar chambers. Some of the roots on the side of the mesh-collar chamber walls seemed to be relatively young eight months after the collars were installed. After girdling *Eucalyptus grandis x urophylla*, Binkley et al (2006) did not observe a drop in soil respiration to the magnitude that was witnessed in a similar experiment in a boreal pine forest (Bhupinderpal-Singh et al. 2003, Hogberg et al. 2001). Fine root biomass and respiration was identified as

the cause for this abnormally low reduction in soil respiration. Owing to large stores of carbohydrates in roots, typical to *Eucalyptus* species (Williams and Woinarski 1997), there was no decrease in either root biomass or root respiration for up to five months after girdling. It seems, therefore, that if mesh-collar chambers are used to isolate the components of soil respiration a longer time period should be employed before expecting an effect. Alternatively, the collars could be installed at the seedling stage of the tree life in order to ensure that the roots are kept out of the collars. The later would also eliminate the possibility that root decomposition will fuel heterotrophic respiration.

The conditions at the HFE sites played a role in the lack of effect of the mesh-collar chambers. The soil at HFE is a poor water retainer and half of the trees are submitted to a drought experiment. Roots were found at depths greater than the lower part of the mesh-collar chambers with the mini-rhizotron (Figure 2.5) and were suspected to grow below 1.5 m depth (Duursma et al. 2011), hence contributing to respiration measured from mesh-collar chambers.

#### *2.4.4 Whole-tree chamber effect*

Greater soil respiration in the control plots than in the WTC as recorded in March and September 2008 is consistent with the observation by Niinisto et al (2004) in their closed chamber experiment. However, over the four years of their experiment, there was no reversal of the trend of greater soil respiration in the control plots than in the ambient WTC to lower respiration in the control plots than in the WTC.

Soil respiration is linked positively to the temperature measured at the soil surface (Bronson et al. 2008, Niinisto et al. 2004, Pajari 1995, Rustad et al. 2001) as well as soil moisture (Keith et al. 1997, Orchard and Cook 1983, Tang et al. 2006). The respective impact of each of these factors on the soil CO<sub>2</sub> efflux might change according to the seasons: soil moisture can become a limiting factor in summer whereas temperature would be a limiting factor in winter. Measurements of soil temperatures (results not presented) showed that for both the September and October measurements, soils in the outside control plots were on average 1.4 °C warmer than in the ambient WTC, a significant difference, whereas the difference ranged from a 0.1 °C cooler to a 0.9 °C warmer for the other months. Except in May, soil moisture was always lower in the control than the WTC plots. The WTC experimental design at HFE has an effect on soil conditions that varies between seasons; this could explain the observed switch from greater to lower soil respiration in the control plots compared to ambient WTC.

#### *2.4.5 Spatial variability in soil respiration*

Rates of respiration recorded in March 2009 using the whole underfloor of the WTC were much greater than rates measured with static chambers. A test in February 2009 using both techniques revealed that on average measurement using the whole underfloor of the WTC were 38.5% greater than measurements using static chambers placed on soil collars. The greater rates can be explained by the spatial heterogeneity in respiration rate depending on the distance from the stem of the tree. There is an exponential increase along a radius from between 1.5 and 1 m to the base of the tree (see Baldocchi et al. 2006, Saiz et al. 2006). Collars were placed at 0.95 metres from

the base of the trees. They were thus unable to detect the fastest rates of respiration. Moreover, the removal of the soils from the WTC and control plots revealed that roots had accumulated along the root barrier that prolonged the walls of the WTC. Respiration rates could therefore be greater along a narrow strip at the edge of the WTC adding up to the respiration measured in March 2009 from the whole underfloor of the WTC.

### ***2.5 Conclusion***

Increased atmospheric [CO<sub>2</sub>] did not induce significant changes in soil respiration from a *Eucalyptus saligna* plantation. However, drought led to a significant reduction in soil respiration compared to an irrigated treatment. Considering predictions of increasing dry spells in future in Australia, soil respiration may be predicted to decline; however, irregular and intense rainfall events may buffer the effect of drier weather by stimulating sudden and intense releases of soil CO<sub>2</sub>. Because of the chamber effect and the relatively short duration of the present study, extrapolation of results to field and ecosystem scales should be undertaken with caution. Long-term studies are needed to better understand the future effect of climate change on soil respiration.

## *Chapter 3*

### *The tracing of carbon through trees and soil from leaves to atmosphere: a pulse labelling experiment*

#### *3.1 Introduction*

Recent studies taking advantages of novel techniques such as girdling and/or the use of stable carbon isotopes have shown that the autotrophic component of soil respiration plays a major part in the total respiration of forest soils. In mature forests, it is common to observe a rhizosphere contribution of more than 50% to total soil respiration and up to 73% has been observed in eucalyptus plantations (Table 1.1 Chapter 1, Giardina et al. 2004). Most of the CO<sub>2</sub> that is released by roots into the soil comes from recently metabolised photosynthates, although the timing (“speed of link”) between aboveground assimilation and soil respiration is a matter of debate (Mencuccini and Höltää, 2010, Kayler et al., 2010). Högberg et al (2001) for instance observed that within five days of a large scale girdling of 20 metre tall boreal pine trees, respiration rates were reduced by up to 37%. Experiments measuring the natural abundance of <sup>13</sup>C in CO<sub>2</sub> have confirmed the link between photosynthates and root respiration (Ekblad et al. 2005, Ekblad and Högberg 2001). The pulse labelling of trees with <sup>13</sup>CO<sub>2</sub> or <sup>14</sup>CO<sub>2</sub> has brought a new resolution to the mapping of carbon allocation within trees and their ecosystems. It allows tracing the fate of carbon through trees from absorption at the canopy level to storage in tissues and release into soils or the atmosphere (Carbone et al. 2007, Hogberg et al. 2008, Subke et al. 2009, Warren et al. 2012).



Pulse labelling also provides a means of studying how each component of respiration, particularly root respiration, will react to future changes in climate. A number of studies have applied pulse labelling to grasslands *in situ* (Bahn et al. 2009, Johnson et al. 2002) or seedlings of trees in pots (Phillips and Fahey 2005) and non-mature trees in mesocosms (Ruehr et al. 2009). However, the physiology of seedlings or saplings and mature trees are often different and transposing the results obtained in the laboratory to full scale trees or forest ecosystems is problematic. Recently, several field based experiments have measured C allocation at the tree and plot scales (Carbone et al. 2007, Hogberg et al. 2008, Subke et al. 2009). When coordinated with climate change experiments such as elevated atmospheric [CO<sub>2</sub>], drought/irrigation or elevated temperature treatments, the impact of these factors can be observed on C allocation to the various compartments of trees and to the CO<sub>2</sub> efflux of both above and below ground tissues (Ruehr et al. 2009).

As stated above, different labels can be used separately or in combination, and low level <sup>14</sup>C (Carbone et al. 2007) or dual labelling of <sup>14</sup>C and <sup>13</sup>C have been applied (Carbone and Trumbore 2007). <sup>14</sup>C offers a greater resolution than <sup>13</sup>C and is more suitable for long term tracing of C. However, being a radioactive component, it is more challenging to manipulate and more costly to analyse. Högberg et al (2008) have demonstrated that short period labelling of 14 year old *Pinus silvestris* with >95 atom% <sup>13</sup>CO<sub>2</sub> could be successfully applied to tracing carbon through the plant into the soil.

Here I present the results of a  $^{13}\text{CO}_2$  pulse labelling experiment in whole tree chambers (WTC) at the Hawkesbury Forest Experiment (HFE, see Chapter 2) that offers the advantage of studying small trees rather than seedlings or small saplings in a greenhouse setting. The initial aim of this pulse labelling was to study the influence of drought and  $[\text{CO}_2]$  on the speed at which carbon travels through *Eucalyptus saligna* trees and is respired belowground.

### **3.2 Materials and methods**

#### *3.2.1 Field site and plant and gas sampling*

The pulse labelling experiment took place at the Hawkesbury Forest Experiment in Richmond, Australia. The HFE examined the effect of elevated atmospheric  $[\text{CO}_2]$  and disruption in water availability (drought) to the growth and functionality of Sydney blue gums (*Eucalyptus saligna*; see Chapter 2 for details) and associated soil processes. Briefly, one-month old Sydney blue gums (*Eucalyptus saligna*) were planted in May 2007, 2.6 metres apart along rows separated by gaps of 3.85 metres. Twelve whole tree-chambers (WTC; 3.25 m diameter and 8.15 m tall) were installed, each growing one *Eucalyptus saligna* under a controlled environment where atmospheric  $[\text{CO}_2]$  and water delivery to the soil were altered in a factorial design with three replicates of each combination of water and  $\text{CO}_2$  treatment: ambient  $[\text{CO}_2]$ -dry, ambient  $[\text{CO}_2]$ -irrigated, elevated  $[\text{CO}_2]$ -dry and elevated  $[\text{CO}_2]$ -irrigated. Since the elevated atmospheric  $[\text{CO}_2]$  treatment did not deliver a significant effect on soil respiration, while the drought treatment did (see Chapter 2), only the ambient  $[\text{CO}_2]$  WTC were used for this pulse labelling experiment. A PVC floor situated 0.45 m above ground and sealed around the stem of the tree separated the soil

compartment from the above ground compartment. The chamber walls were buried into the soil just outside of a root barrier that extended to a depth of 1 m. The underfloor compartment was constantly ventilated but could also be sealed when needed. At the time of the pulse labelling, the droughted trees had not received any water for more than three months but were rewatered on 4 March 2009, four days before the end of the pulse chase experiment.

At around midday on 10 February and 11 February 2009, after ensuring the WTC did not present any leak, the six ambient [CO<sub>2</sub>] chambers were pulse labelled with 5 litres of 99 atom% <sup>13</sup>CO<sub>2</sub> (WTC 1, 5, 3 and 7 on 10 February, WTC 7 and 11 on 11 February). The ventilation of the top compartment of the WTC was shut before introducing the <sup>13</sup>CO<sub>2</sub>. The atmospheric [CO<sub>2</sub>] inside the chamber was left to draw down until the compensation point was reached (at which absorption of CO<sub>2</sub> for photosynthesis was balanced by the release of CO<sub>2</sub> through respiration). The [CO<sub>2</sub>] within the WTC was monitored with both an inbuilt WTC gas analyser and a portable CO<sub>2</sub> gas analyser Vaisala GMP342 (Vaisala, Finland). When the [CO<sub>2</sub>] stabilised 1.5 to 2 minutes after introduction of the label, three air samples were taken through the wall of the WTC with a syringe and fine needle. Another two series of three samples were taken at the estimated mid-point of the draw-down (20 to 60 minutes after pulse labelling depending on WTC) and again when the compensation point was reached (1.5 to 2.5 hours after pulse labelling depending on WTC). For each sample, 12 ml of gas was extracted and immediately transferred to an evacuated Exetainer tube.

The pulse was then traced for a month through leaves, roots and soil CO<sub>2</sub> effluxes. First of all, two fully expanded leaves were collected from each tree 24 hours before the pulse and then immediately at the end of the draw down (time point 0) and 1, 2, 4, 8, 12, 18, 24, 48, 72, 144, 480, 552, 576 hours after the pulse. They were frozen immediately at -80°C before being freeze-dried and ground in a Retsch ball mill (MEP instruments, North Ryde, Australia). Samples were stored in desiccators until further analysis.

Soil samples were collected 24 hours before and then 2, 4, 7, 20, 23, 27 and 34 days after the pulse labelling. Four samples per WTC were collected with a 5-cm auger at three depths: 0-10 cm, 10-30 cm and 30-70 cm. Roots were extracted from soil samples by wet sieving (>250 µm) and separated into root tips (using a microscope), fine roots (<1 mm in diameter) and coarse roots (>1 mm in diameter). All root samples were immediately frozen at -80°C before being freeze-dried. Fine and coarse roots were ground to a powder in a Retsch ball mill, and all samples were kept in desiccators before further analysis.

A Keeling plot approach was used in order to estimate the  $\delta^{13}\text{C}$  of soil-respired CO<sub>2</sub>. Gas sampling of soil respiration was conducted using the whole underfloor space of the WTC after shutting down the ventilation system and blocking ports, thus creating a large size static chamber (~3.7 m<sup>3</sup>). A Vaisala GMP342 CO<sub>2</sub> analyser was connected to the inside of the under floor compartment of the WTC and controlled from the outside to measure the rate of soil respiration. A computer fan was installed to circulate air in direction of the Vaisala and gas sampling spot in order to insure

optimum air mixing. When sampling commenced, the under floor fan of the WTC was turned off, the computer fan turned on and the air vents of the WTC shut. The door of the above floor part of the WTC was kept ajar in order to eliminate any pressure difference between the two compartments. Gas samples (12 ml) were then taken through the outer wall of the WTC, the first one immediately after conducting the above operation. Another three samples followed at an interval of 10 minutes. The fifth and last sample was taken when the underfloor [CO<sub>2</sub>] reached 1000 ppm or above. Each sample was immediately transferred to a 12 ml evacuated Exetainer tube. Blue Tack was applied to the top of Exetainer tubes to prevent any leakage or exchange and associated fractionation during storage before  $\delta^{13}\text{C}$  analysis. Small needle gauges were chosen in order to avoid large holes in the WTC walls and Exetainer septa and hence limit any future leakage. The precise time at which each sample was taken and the [CO<sub>2</sub>] in the below floor compartment of the WTC as displayed by the Vaisala data logger were both recorded.

A first sampling of soil respiration was conducted 24 hours prior to pulse labelling. Starting five hours after pulse labelling, samples were collected every six hours for the next seven days. The intensity of sampling was then reduced to twice a day for two days and then once a day for three days. Finally there were three more samplings three days apart ending 3 March inclusive. As the droughted plots were watered on 4 March, a new round of sampling was started from 5 March with three sampling events for this day, two for the following day and one sampling per day on the next four days. Three samples from a standard CO<sub>2</sub> in air gas bottle were also taken on

each day of sampling in order to account for any exchange of gas through the septum of the Exetainer tubes resulting in a possible  $^{13}\text{C}$  shift during storage.

### 3.2.2 Isotope analysis and theory

All samples were analysed on a DeltaV Advantage isotope ratio mass spectrometer (IRMS), coupled to a FlashHT and ConFlo IV (Thermo Fisher Scientific, Bremen, Germany). Ground leaf (0.5 mg), root tissue and of whole root tips (0.4 mg) were weighed into tin capsules for  $\delta^{13}\text{C}$  analysis. Isotopic values are expressed in  $\delta$  notation (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard, following the formula:

$$\delta = \frac{R_{\text{sample}}}{R_{\text{standard}} - 1} * 1000$$

where R is the ratio of the  $^{13}\text{C}$  to  $^{12}\text{C}$  in the sample and standard respectively.

The amount of  $^{13}\text{CO}_2$  taken up by the trees during the pulse labelling was estimated with the difference in  $^{13}\text{CO}_2$  measured immediately (1.5 to 2 minutes) after introduction of the labelled  $\text{CO}_2$  and the amount of  $^{13}\text{CO}_2$  measured at compensation point at the end of the labelling period. First of all, the  $\delta^{13}\text{CO}_2$  of the air samples taken during pulse labelling was converted to  $^{13}\text{C}$  atom percent values following the equation:

$$^{13}\text{CO}_2 \text{ atom \%} = \frac{100 * 0.0111802 * \left( \frac{\delta_{\text{sample}}}{1000} \right) + 1}{1 + 0.0111802 * \left( \frac{\delta_{\text{sample}}}{1000} \right) + 1} \quad (1)$$

where 0.0111802 is the standard value of  $^{13}\text{C}/^{12}\text{C}$  ratio of VPDB.

Then, in order to calculate the amount of  $^{13}\text{CO}_2$  present in the upper compartment of the WTC:

$$m(^{13}\text{C}) = ^{13}\text{CO}_2 \text{ atom \%} * m(\text{CO}_2)$$

where  $m(^{13}\text{C})$  is the amount of  $^{13}\text{C}$  in mg and  $m(\text{CO}_2)$  is the amount of  $\text{CO}_2$  in mg measured with the Vaisala GMP 342 at time of sampling.

The amount of  $^{13}\text{CO}_2$  taken up by the trees was then:

$$m(^{13}\text{C}) \text{ taken up} = m(^{13}\text{C}) \text{ start pulse} - m(^{13}\text{C}) \text{ end pulse.}$$

Equation 1 was used to calculate the atom percent of each sample of soil respiration as the basis to determine the amount of  $^{13}\text{C}$  that went through the trees and the soil and was released as  $^{13}\text{CO}_2$  into the atmosphere through soil respiration. To estimate the amount of  $^{13}\text{CO}_2$  in soil respiration that originated from the pulse labelling (excess  $^{13}\text{C}$  respired), expressed as  $\text{mg}^{13}\text{CO}_2 \text{ m}^{-2}$ , the amount of  $^{13}\text{CO}_2$  occurring naturally ( $^{13}\text{CO}_2$  background in atom%) and measured from the pre-pulse sampling was subtracted to the amount of  $^{13}\text{CO}_2$  measured in the sample at any given time ( $^{13}\text{CO}_2$  sample in atom%) and the difference multiplied by the respiration rate at this time following the equation:

$$\text{Excess } ^{13}\text{C} \text{ respired} = \frac{(^{13}\text{CO}_2 \text{ sample} - ^{13}\text{CO}_2 \text{ background})}{100} * (R_S * S_{\text{WTC}} * T) \quad (2)$$

where  $R_S$  is the soil  $\text{CO}_2$  effluxes ( $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) calculated as the average of the rates of soil respiration at the time the soil efflux samples were taken and the rate of respiration recorded during the previous sampling.  $S_{\text{WTC}}$  is the basal area of the WTC ( $8.295768 \text{ m}^2$ ) and  $T$  is the elapsed time between sampling (in hours).

The mean residence time and half life of the introduced  $^{13}\text{C}$  through the tree and soil were calculated following the exponential decay formula used by Ruehr et al (2009):

$$N(t) = N_0 e^{-\lambda t} \quad (3)$$

Where  $N(t)$  is the quantity of  $^{13}\text{C}$  after time  $t$ ,  $N_0$  is the quantity of  $^{13}\text{C}$  at the peak of  $^{13}\text{C}$  corresponding to time  $t=0$ ,  $\lambda$  is the decay constant and  $t$  is the time in days. The mean residence time was derived from this equation as  $\tau = 1/\lambda$  and the half life is  $\tau_{1/2} = \ln(2)/\lambda$ . The fit of the model was verified by calculating the correlation between values of  $N(t)$  calculated with the model and the  $N(t)$  values measured from samples of soil respiration.

Hereafter  $\delta^{13}\text{C}_{\text{leaf}}$  designates the  $\delta^{13}\text{C}$  signature of leaves of the pulse labelled trees,  $\delta^{13}\text{C}_{\text{roots}}$  designates the  $\delta^{13}\text{C}$  signature of roots of pulse labelled trees and  $\delta^{13}\text{C}_{\text{RS}}$  the  $\delta^{13}\text{C}$  signature of soil respired  $\text{CO}_2$ .

### **3.3 Results**

Immediately after introduction of the labelled  $\text{CO}_2$ , the  $[\text{CO}_2]$  in the aboveground compartment of the WTC was ranged from 368 to 416ppm - close to the normal



atmospheric concentration for this site in the middle of the day. The  $\delta^{13}\text{C}$  signature was, on average, just under 13000‰ immediately after introduction of the  $^{13}\text{CO}_2$ , over 8800‰ at mid draw-down and about 1900‰ at compensation point (Figure 3.1). Depending on the WTC, it took between 80 and 150 minutes to reach the compensation point, however there was no relationship between  $\text{CO}_2$  uptake and watering treatment, as  $\text{CO}_2$  draw-down in the watered WTC 3 and 11 were comparable to the droughted WTC 5 and 9 but all differed from WTC 1 and 7 (Figure 3.1). Moreover, a similar amount of  $^{13}\text{CO}_2$  was taken up by the trees regardless of the watering treatment they received (Table 3.1). The  $^{13}\text{CO}_2$  that was absorbed by the trees during labelling amounted to just over 50% of what was introduced in each WTC.

Table 3.1.

Amount of  $^{13}\text{CO}_2$  taken up by trees during pulse labeling and recovered into soil respiration during pulse chase after 9723 mg of  $^{13}\text{CO}_2$  were introduced in each WTC. Result in mg of  $^{13}\text{CO}_2$  plus/minus standard error

WTC	$^{13}\text{CO}_2$ (in mg) taken up by trees	$^{13}\text{CO}_2$ (in mg) recovered from soil respiration after 10 days	$^{13}\text{CO}_2$ (in mg) recovered from soil respiration during the pulse chase
Irrigated	4633 $\pm$ 426	1302 $\pm$ 180	1562 $\pm$ 239
Droughted	4966 $\pm$ 424	872 $\pm$ 85	1314 $\pm$ 112

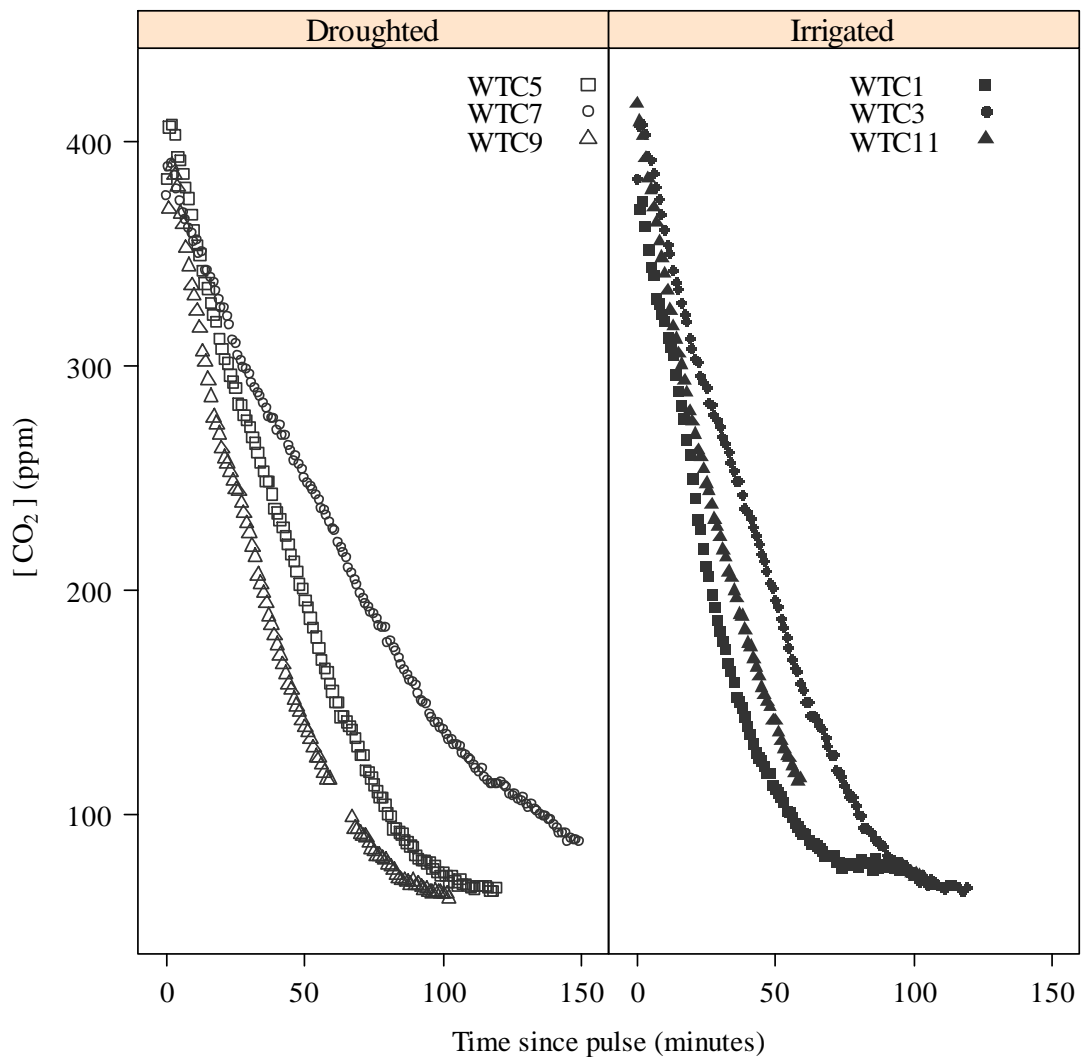


Figure 3.1.

Draw-down after introduction of 5 l of <sup>13</sup>CO<sub>2</sub> in the whole tree chambers at HFE in the droughted and irrigated treatments.

$\delta^{13}\text{C}_{\text{leaf}}$  peaked immediately (at the end of the draw-down) in two out of the three irrigated WTC but took up to two hours to peak in two of the three droughted chambers (Figure 3.2).  $\delta^{13}\text{C}_{\text{leaf}}$  dropped rapidly back to pre-pulse values in all the WTC within 48 to 72 hours. The decrease mostly followed a typical decay function for the leaves of the irrigated WTC. In droughted chambers, however, a new peak appeared clearly between 12 and 24 hours after the pulse labelling corresponding to the period between the middle of the night and the middle of the day. From 48 to 72

hours post-pulse labelling,  $\delta^{13}\text{C}_{\text{leaf}}$  declined gradually over the next 21 days, marking the end of the pulse chase in the leaves. For the last three leaf samplings,  $\delta^{13}\text{C}_{\text{leaf}}$  returned to pre-pulse values in the irrigated WTC. In droughted WTC,  $\delta^{13}\text{C}_{\text{leaf}}$  remained above its pre-pulse level.

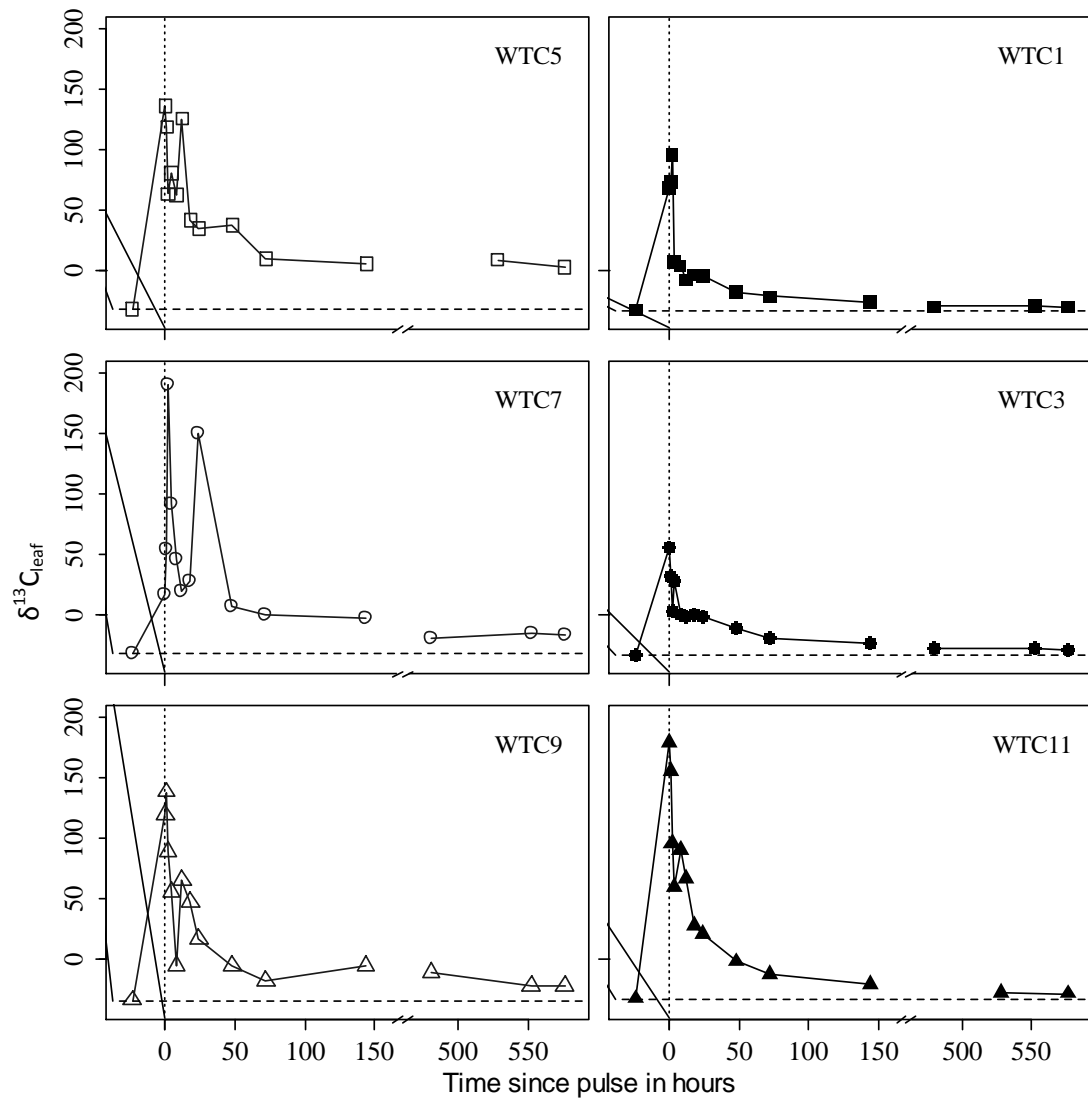


Figure 3.2.

$\delta^{13}\text{C}$  of *E. saligna* leaves during the pulse chase period. The vertical dotted line represents the pulse labeling and the horizontal dashed line is the pre-pulse value  $\delta^{13}\text{C}$  for each tree. Light markers indicate droughted WTC, dark markers are irrigated WTC.

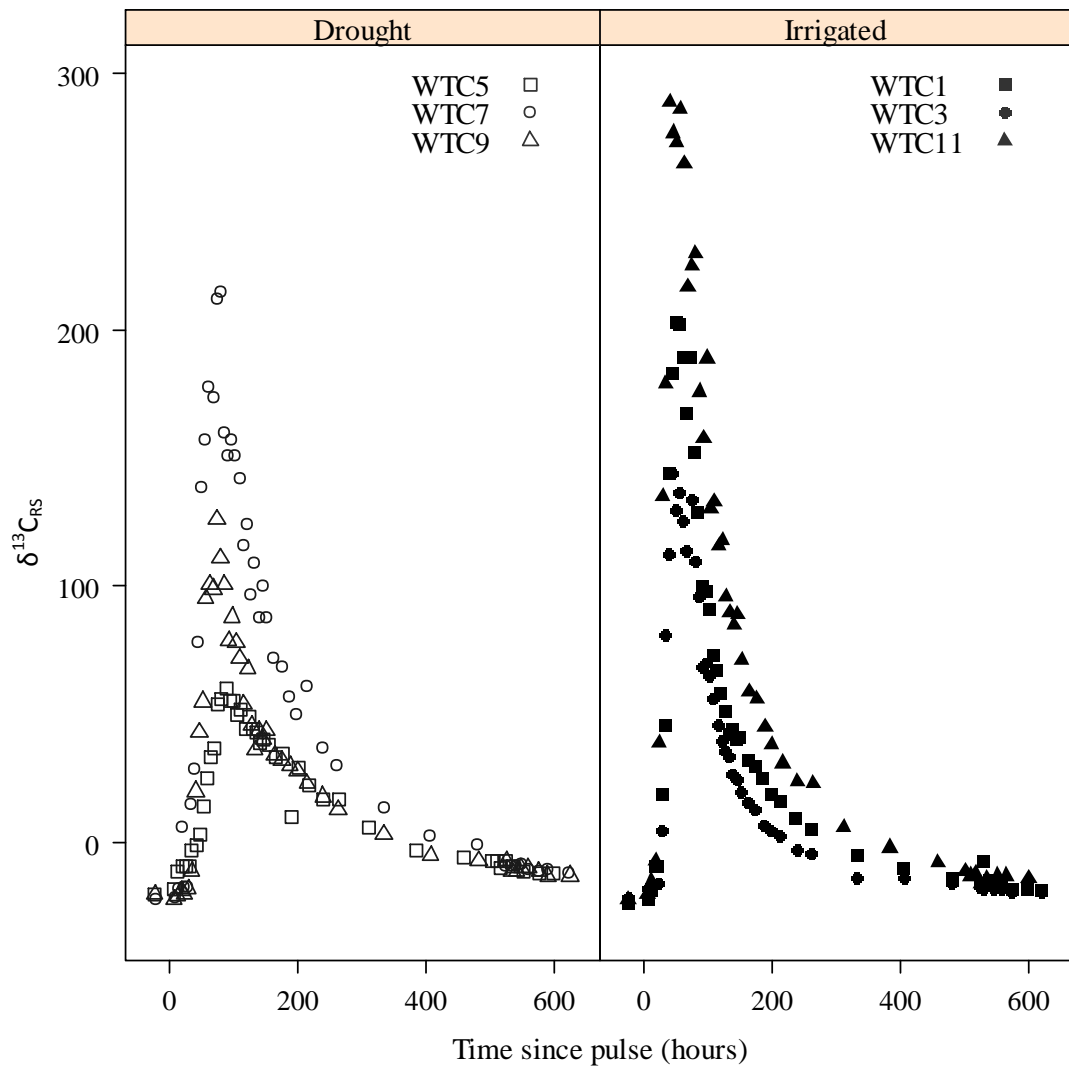


Figure 3.3.

Evolution of the  $\delta^{13}\text{C}$  values of soil respiration ( $\delta^{13}\text{C}_{\text{RS}}$ ) of each of the labeled WTC during the 26 day pulse-chase.

In the soil  $\text{CO}_2$  efflux,  $\delta^{13}\text{C}_{\text{RS}}$  peaked between 40 and 52.5 hours (approximately two days) after pulse labelling in the irrigated WTC (Figure 3.3).  $\delta^{13}\text{C}_{\text{RS}}$  reached a peak between 73 and 87 hours (three to four days) after pulse in the soil respiration of the droughted WTC. An increase in  $\delta^{13}\text{C}_{\text{RS}}$  in WTC 1, 3, 5 and 7 could be observed as soon as six hours after the pulse labelling. It took 18 hours for WTC 9 and 11 to show the same trend. There were large variations in the size of  $\delta^{13}\text{C}_{\text{RS}}$  peaks both within and across treatments. The mean of the value for the peaks of  $\delta^{13}\text{C}_{\text{RS}}$  for

irrigated WTC was 212‰, greater than that of the droughted WTC at 133‰. However, the second highest peak was that of a droughted WTC (WTC 7) at 214‰. There was a strong correlation between the value of the peak of  $\delta^{13}\text{C}_{\text{leaf}}$  and the peak of  $\delta^{13}\text{C}_{\text{RS}}$  with a coefficient of correlation equalling 0.926 for the droughted trees and 0.986 for the irrigated trees.

The excess  $^{13}\text{C}$  label could be measured in the soil  $\text{CO}_2$  effluxes for a longer period of time after pulse labelling in the droughted WTC than the irrigated chambers. The mean residence time of the label in the soil respiration of the droughted WTC was more than eight days whereas it was approximately six days in the irrigated WTC (Table 3.2). This difference was not significant (t test,  $P>0.05$ )

Table 3.2.

Mean residence time and half life of the  $^{13}\text{C}$  label in the soil  $\text{CO}_2$  effluxes as calculated by equation 3

Treatment	Mean residence time in days ( $\tau$ )	Half life in days ( $\tau_{1/2}$ )
Droughted	$8.2 \pm 0.7$	$5.7 \pm 0.5$
Irrigated	$6.1 \pm 0.5$	$4.2 \pm 0.3$

The pre-pulse  $\delta^{13}\text{C}_{\text{RS}}$  ranged from -20.8‰ to -24.2‰ for the irrigated WTC and -21.9‰ to -23.5‰ in the case of the droughted WTC. By the last sampling date, one month after labelling, none of  $\delta^{13}\text{C}_{\text{RS}}$  of any of the WTC was back to the pre-pulse values. The  $\delta^{13}\text{C}_{\text{RS}}$  of the irrigated WTC were the closest to normal at the end of the

pulse chase, between 2‰ and 8.3‰ above the pre-pulse values, whereas droughted WTC were 8.4‰ to 11.7‰ above their pre-pulse values.

The rewatering of the droughted chambers on 4 March did not result in a change in the general pattern of the evolution of the  $\delta^{13}\text{C}_{\text{RS}}$  from these WTC.

Over the sampling period following the pulse labelling, an average of approximately  $1.31 \pm 0.11$  g and  $1.56 \pm 0.24$  g of  $^{13}\text{CO}_2$  was recovered from the soil  $\text{CO}_2$  effluxes of respectively each of the droughted and irrigated WTC (Table 3.1). This represents 26% and 34% of the amount of  $^{13}\text{CO}_2$  that was taken up by the trees during pulse labelling. The mean residence time and half life of the label in soil respiration were already different between droughted and irrigated trees ten days after the pulse labelling: only  $0.87 \pm 0.08$  g of  $^{13}\text{CO}_2$  had been emitted from the soils of the droughted WTC whereas  $1.30 \pm 0.18$  g of  $^{13}\text{CO}_2$  had been emitted from the irrigated WTC, accounting for 18% and 28% respectively of the  $^{13}\text{CO}_2$  that was taken up by trees during labelling. The difference in amount of  $^{13}\text{CO}_2$  emitted from the soils of the droughted WTC and irrigated WTC was however not significant at any time (ANOVA,  $P > 0.05$ ).

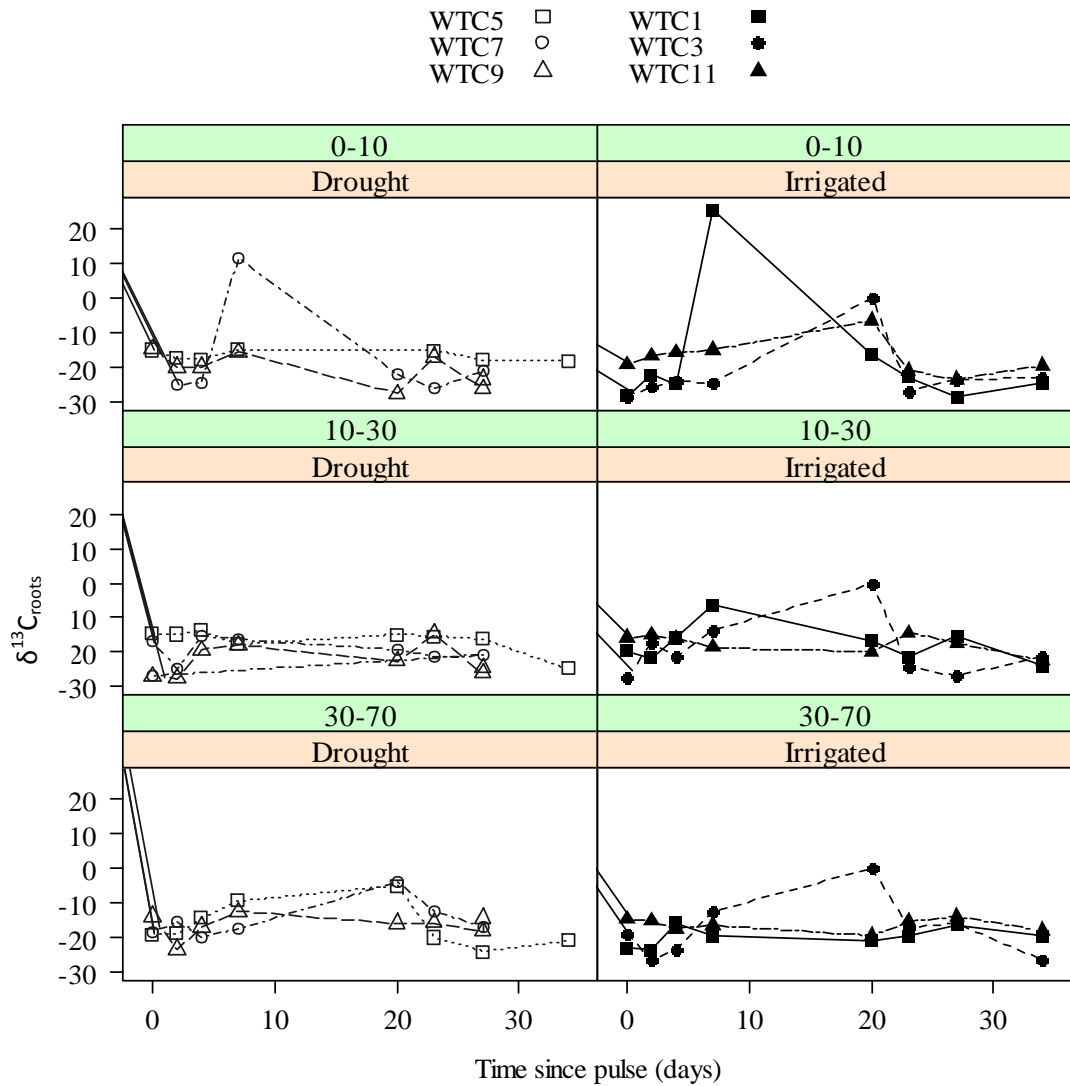


Figure 3.4.

Evolution of  $\delta^{13}\text{C}_{\text{roots}}$  in the root tips collected at three different depths for each WTC and treatment during the 34 day pulse chase.

Before pulse labelling,  $\delta^{13}\text{C}_{\text{roots}}$  values were highly variable across WTC. Regardless of the treatment and depth they ranged from -15‰ to -30‰ (Figure 3.4 and 3.5). After labelling, there was no clear pattern or peak of labelled C concentration across watering treatments. Within a given WTC, root type (ie root tips, root <1mm diameter and root >1mm diameter) and soil depth produced clear patterns. Coarse roots (>1mm)  $\delta^{13}\text{C}$  between day 4 and day 7 after pulse labelling followed that of the fine roots (<1mm) between day 2 and 4 after pulse labelling. Most of the time,

$\delta^{13}\text{C}_{\text{roots}}$  stayed within the values of -30‰ and -15‰. The greatest  $\delta^{13}\text{C}_{\text{roots}}$  were measured in root tips.

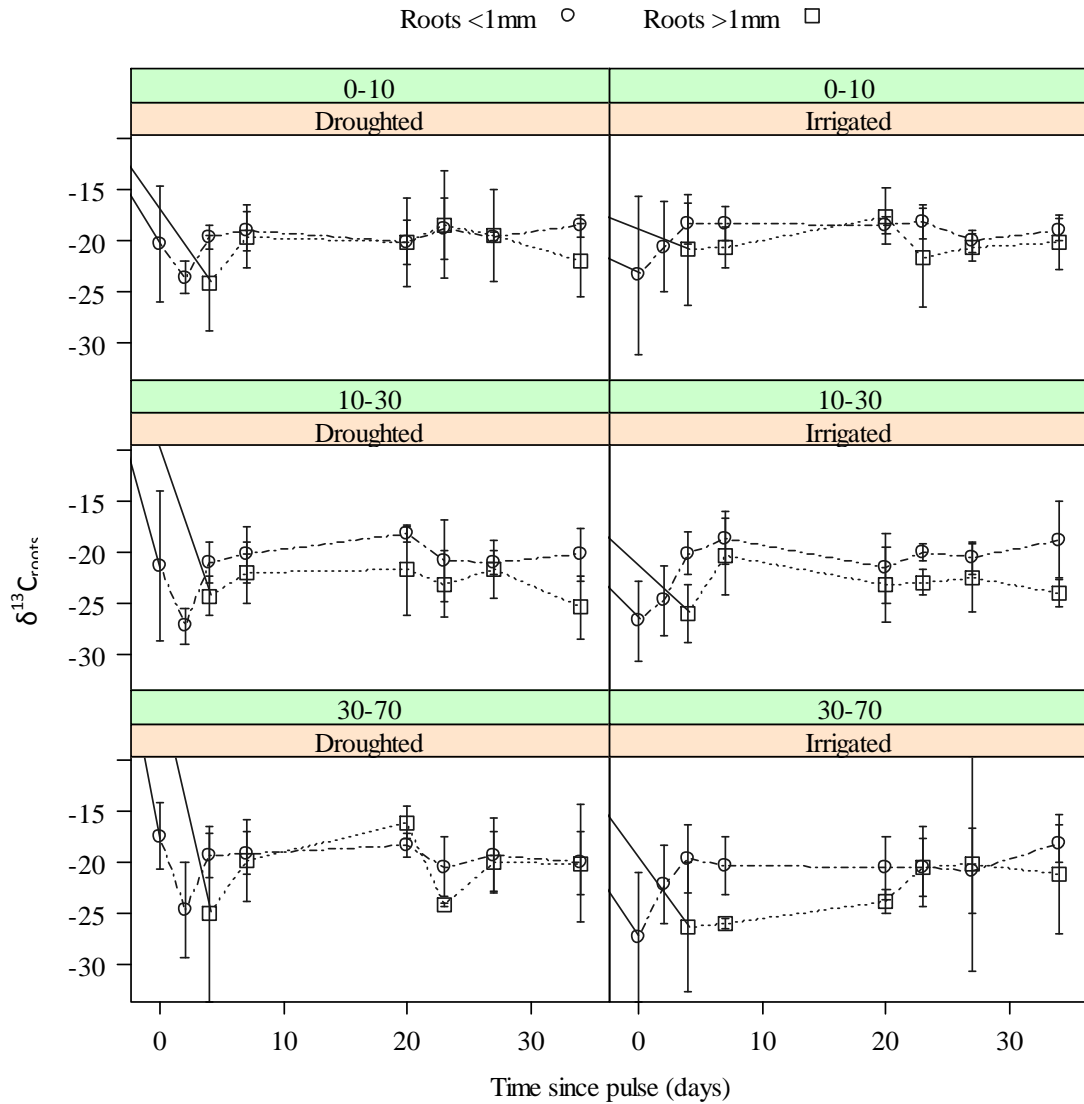


Figure 3.5.

Evolution of  $\delta^{13}\text{C}_{\text{roots}}$  in the fine roots (<1 mm) and coarse roots (>1 mm) collected at three different depths during the 34 day pulse chase. Results are averaged over three WTC for each treatment.



### **3.4 Discussion**

The present results show that the delay between pulse labelling and the peak of  $\delta^{13}\text{C}_{\text{RS}}$  was governed by the velocity at which photosynthates travelled from the *E. saligna* canopy to their belowground compartments. It took only 6 to 18 hours to observe an increase of  $\delta^{13}\text{C}_{\text{RS}}$  and less than 48 hours, on average, for  $\delta^{13}\text{C}_{\text{RS}}$  to peak in the irrigated trees. More time, 80 hours approximately, was required to observe peaks of  $\delta^{13}\text{C}_{\text{RS}}$  in droughted trees. Drought has been shown to slow the transfer of recently metabolised carbohydrates from the canopy to the belowground compartment of trees (Ruehr et al. 2009). A second factor that may have been involved at the HFE is root distribution. Based on their water intake, droughted trees were suspected to have roots growing deeper than irrigated trees (Duursma et al. 2011). This difference in the depth of root increases the distance the labelled C has to travel through the droughted trees compared to the irrigated ones before being released into the soil by root respiration. However, for both irrigated and droughted trees and considering that the trees were up to 10 metre tall at HFE, the time lag between pulse and the maximum enrichment in  $\delta^{13}\text{C}_{\text{RS}}$  was short. The literature reports lags ranging from two to four days for trees half the size of the *E. saligna* at HFE (Carbone et al. 2007, Hogberg et al. 2008, Horwath et al. 1994, Subke et al. 2009). Depending on the season, pulse labelling on 8 to 9 metre tall beech trees showed a lag of 64 to 84 hours before the peak of  $^{13}\text{CO}_2$  was observed in the soil respiration (Plain et al. 2009). Air temperature is a key driver of phloem velocity and has previously been argued to explain the variations in delay between pulse and the peak of  $\delta^{13}\text{C}$  in soil respiration (Plain et al. 2009). Temperatures were uncharacteristically low during the pulse chase experiment at HFE which suggests that the increase in  $\delta^{13}\text{C}_{\text{RS}}$  could have started earlier under warmer air temperature.

There was no peak of  $\delta^{13}\text{C}_{\text{roots}}$  in the roots and only a general increase in  $\delta^{13}\text{C}_{\text{roots}}$  of root tips. There was a great variability in the labelling of the roots within treatment and with depth of the roots. In agreement with the present results, Högberg et al (2008) only found a slight increase of  $\delta^{13}\text{C}$  of 2 to 3 ‰ in the ectomycorrhizal root tips of pulse labelled *Pinus silvestris* and the amount of label in roots was heterogeneous. Newly photosynthesized carbon in both grass and trees is sent in priority to the growing compartments of plants such as the root tips (Phillips and Fahey 2005, Thornton et al. 2004). In contrast to the quick change in  $\delta^{13}\text{C}$  observed in the root tips of *Lolium perenne*, larger and older roots showed only little change when air supply was switched from atmospheric origin to a  $^{13}\text{C}$ -enriched source (Thornton et al. 2004). Because recent assimilates are transported in soluble forms that can diffuse easily through membranes (Phillips and Fahey 2005, Thornton et al. 2004) and because they are transported to growth areas where membranes are more porous to solute (Thornton et al. 2004), a major part of the labelled C is released directly into the soils. Only a small part of newly photosynthesized C stays in the root tips, being assimilated in tissues that will become part of the thicker roots once the roots are elongating. As the label is retained in fine roots that will grow to coarser roots, there is a delay in  $\delta^{13}\text{C}_{\text{roots}}$  evolution between the fine and coarse roots such as the one observed between day 4 and day 7 following the pulse labelling. The combination of an absence of true peak of  $\delta^{13}\text{C}_{\text{roots}}$  but a clear peak in  $\delta^{13}\text{C}_{\text{RS}}$  at HFE indicates that most of the labelled C went through the roots and passed directly into the soil as a product of respiration. Högberg et al. (2002) demonstrated that in a boreal forest, 75% of the C allocated to roots was respired. The residence time of recently photosynthesized C in the root tips must therefore be very short. Because,

the sampling regime for the roots was not as intensive as the one for soil CO<sub>2</sub> effluxes, a peak of  $\delta^{13}\text{C}_{\text{roots}}$  may have appeared on the second day after the pulse labelling but could have been missed by either sampling on Day 1 or Day 3 after pulse labelling if it was of a short duration.

Methodologically, the structure of the WTC presented a number of benefits in conducting a <sup>13</sup>CO<sub>2</sub> pulse labelling experiment. First of all, the floor of the WTC had the advantage of isolating the soil compartment from the labelling of the canopy. This type of separation is easily achievable in laboratory experiment using smaller trees or plants. In the field however, on large scale pulse labelling, it is not always possible to separate the above ground compartment and soil compartment (Bahn et al. 2009, Hogberg et al. 2008, Subke et al. 2009). The non isolation of soil from the canopy compartment results in observing an immediate increase of  $\delta^{13}\text{C}$  in the soil CO<sub>2</sub> efflux that lasts for 48 to 72 hours after pulse labelling (Hogberg et al. 2008, Subke et al. 2009). This initial increase in  $\delta^{13}\text{C}$  originates from the penetration of the label by gas diffusion into soil during pulse labelling. The labelling study at the HFE can be seen as being at the crossroad of laboratory pot experiments using single plants and larger scale field experiments in natural ecosystems. At HFE, a single tree was growing inside each WTC and the soil within the WTC was sprayed to prevent the growth of any grass or woody plants. Moreover, none of the <sup>13</sup>CO<sub>2</sub> was transferred by diffusion into the soil during labelling. Any <sup>13</sup>C in soil CO<sub>2</sub> efflux after labelling, besides that that is naturally occurring, is therefore originating from the roots of the sole *E saligna* growing in the chamber. This confirms that the increase of  $\delta^{13}\text{C}_{\text{RS}}$  that appeared 6 to 18 hours and peaked 48 to 80 hours after pulse originated

from the  $^{13}\text{CO}_2$  that the trees were labelled with and is the product of root respiration. The walls of the WTC were buried into the soil and extended by a one metre deep root barrier preventing most roots from neighbouring trees to penetrate the belowground space of the WTC and induce a dilution of the label. This also ensured that very little, if any, of the label was lost to transport by roots outside of the sampling area. Finally, using the whole underfloor for sampling soil  $\text{CO}_2$  effluxes eliminated the spatial variations in magnitude of  $\delta^{13}\text{C}_{\text{RS}}$  values that sampling using small collars and static chambers are subjected to (Subke et al. 2009). Root distribution was very variable within each WTC as was noted during soil extraction after harvesting of the trees. Collar sampling may have resulted in an approximation of the true value of the peaks of  $\delta^{13}\text{C}_{\text{RS}}$  because the root density might have varied underneath each collar. The current method of sampling the whole underfloor of WTC for soil respiration allowed a more accurate representation of the recent  $^{13}\text{C}$  allocation in soil respiration of each WTC.

### ***3.5 Conclusion***

The  $^{13}\text{CO}_2$  pulse labelling experiment at HFE confirmed the finding of previous study that root respiration is directly linked to recently metabolised photosynthates. This first pulse labelling of Eucalyptus trees has indicated that the pathway of newly synthesized carbohydrates from leaves to roots may be faster in this genus of trees than for others. However the time lag between  $\text{CO}_2$  assimilation by the canopy and soil respiration of the corresponding carbon is increased by drought that reduces the velocity of the transfer of carbon through the plant. More research needs to be done on the effect of the length of the drought period and the potential correlation with the

time lag between the formation of photosynthates in the canopy of trees and the release of the CO<sub>2</sub> to the atmosphere from the belowground compartment.

## *Chapter 4*

### *The recovery of soil respiration post fire: examples of two sites in*

### *South East Australia.*

#### *4.1 Introduction*

Climate change is expected to increase rainfall extremes in already wet areas such as polar and sub-polar regions while dry areas such as part of the sub-tropics would see longer dry periods with shorter and more intense rainfall events. As a consequence to the increase in atmospheric [CO<sub>2</sub>], temperatures could increase by 1.1 to 6.4 °C by 2100 (Solomon et al. 2007). Other possible changes will be region-specific such as, in Australia, an increase in frequency of strong cold fronts that generate strong winds (Hasson et al. 2009). These conditions are likely to create and increase the occurrence of favourable circumstances to the lighting and propagation of wildfires (Adams et al. 2013, Attiwill and Adams 2012, Williams et al. 2001).

On the global scale, the origins of fire can be traced to the appearance of terrestrial plants (Bowman et al. 2009). Fire holds a more central place in Australia's natural and anthropogenic history than in most other parts of the world (Attiwill 1994). Most of the ecosystems of the continent present conditions such as highly flammable vegetation, dry and hot climate, large areas of bushland that are conducive to fires (Attiwill 1994). Indeed, the past hundred years alone were marked by a series of major, large-scale events that, originating from natural causes or human activities, were spread all around the continent from sea level to the Australian Alps. The states

of Victoria (1926, 1939, 1993, 2009), New South Wales (2001-2, 2003), South Australia (1983, 2005) and Tasmania (1967) were all touched by these wildfires. For instance the High Country fire of January-February 2003 affected an area of 1.75 million hectares, removing all plant cover in places (Bear and Pickering 2006), whereas the 'Black Saturday' fire in Victoria in February 2009 was of a lesser extent, burning over 272 000 hectares, but was the most costly in term of human lives (Attiwill and Adams 2012).

Fire has an impact on a great many ecosystem processes and particularly on carbon cycling. The effect of fire on soil respiration is dependent on the nature of the ecosystem, age of the forest stand, species composition and severity of the burn. Immediately after a fire event roots and microbial death will slow soil respiration (Hamman et al. 2007). In the longer term, fire frequency and intensity influence soil respiration by facilitating the rejuvenation of forests. Indeed, total soil respiration is generally greater in older than younger stands (Gough et al. 2007) although, some very old stands show declining respiration (Czimczik et al. 2006).

The contribution of each component of soil respiration to total respiration (heterotrophic or autotrophic) also varies with the frequency of fires. In boreal forests the decomposition of fire residues can create a peak in soil respiration of heterotrophic origins in the months and up to three years following fire (Bond-Lamberty et al. 2004, Schulze et al. 2000). Heterotrophic respiration ( $R_h$ ) is then reduced in younger stands -i.e. 5 to 70 years old (Bond-Lamberty et al. 2004, Czimczik et al. 2006, Gough et al. 2007). However, in warmer climates,  $R_h$  is not

always affected by fire and can thus stay constant in the first years following the burn (Irvine et al. 2007, Meigs et al. 2009). Moreover a quick recovery of root respiration, possibly due to the dense understorey that benefits from the opening in the canopy after fire, can correct the imbalance between autotrophic and heterotrophic respiration within two years (Irvine et al. 2007). Root respiration will also be dependent on the species sensitivity to fire and their ability to recover after a burn.

Eucalyptus trees are often classified as ‘seeders’ when stands are killed by fire and regenerate from seed germination or ‘resprouters’ when trees survive fire and regenerate from epicormic shoots or coppice. Soil respiration originating from roots may vary according to which category the dominant species of a Eucalyptus forest belongs to. Equally the opening of the canopy and consumption of the litter layer following more intense fire sometimes lead to an enrichment in species diversity (Dumas et al. 2007) and/or a shift in species abundance (Meigs et al. 2009). This in turn may be accompanied by a change in net primary production (NPP) that can partly compensate for the loss of aboveground live carbon and thus maintain the rates of soil respiration (Meigs et al. 2009).

Here I focus on the effects of wildfires on soil respiration in natural forests in two regions of South-Eastern Australia. This work addresses the lack of published examinations of the international hypotheses described above with the hypothesis that the soil respiration in Australia is resilient to fire and that respiration of soil supporting ‘resprouters’ will recover more rapidly than that of soils supporting ‘seeders’.



## **4.2 Materials and Methods**

This work was conducted on two different sites in contiguous States of South East Australia at the Mount Disappointment State Forest in Victoria and Snowy Plains in New South Wales.

### **4.2.1 Mount Disappointment State Forest**

The first experimental site is located in the Mount Disappointment State Forest approximately 50 km North of Melbourne, in the state of Victoria, at an altitude of around 800m above sea level. The mean annual rainfall in the area, calculated over the 15 years preceding this study, is 640 mm over an average 91.5 rainy days. Mean minimum temperatures vary from 3.9 °C in July to 12.6 °C in February whereas mean maximum temperatures oscillate between 9.0 °C in July and 24.6 °C in January (BOM).

Three sites were chosen for their differences in tree cover and age class. The first site is dominated by Messmate (*Eucalyptus obliqua*) accompanied with Narrow-leaved Peppermint (*Eucalyptus radiata*) and a bracken fern understorey (*Pteridium esculatum*). It is hereafter referred to as Messmate 1. A second site is a stand of Messmates (*E. obliqua*), Narrow-leaved Peppermints (*Eucalyptus radiata*), Broad-leaved Peppermints (*Eucalyptus dives*) and an understorey of bracken fern (*Pteridium esculatum*). It is referred to as Messmate 2. The final site is almost exclusively covered with Mountain Ash (*Eucalyptus regnans*) and a few Silver Wattles (*Acacia*

*dealbata*) and is therefore referred to as Mountain Ash. Each study site consisted of a square plot of 40 m by 40 m, bordered by a 20 metre buffer from roads or other tree stands.

The soils are sandy clay loam (Udic Ustochrept) at Messmate 1, dark reddish brown gravely sandy clay (Umbreptic Ustoprecht) for the Messmate 2 site and brown earth (Udic Ustochrept) at Mountain Ash of which a detailed description is given in Adams (1984). The pH level is 5.0 at the Messmate 1 site, 5.4 at Messmate 2 and 5.7 under the Mountain Ash forest. In November 2009, total carbon content was 3.3% at Messmate 1, 4.9% at Messmate 2 and 10.7% at the Mountain Ash site.

The 'Black Saturday' bushfires on 7 February 2009 burnt through the sites incinerating the litter layer and understorey and defoliating the crown of the trees. About six months after the fire storm, the first signs of vegetative regrowth were beds of moss scattered over the sites and surrounded by patches of bare soil. Messmates, belonging to the so-called 'resprouters' group of eucalypts, survived the fire storm and resprouted from epicormic shoots at Messmate 1 and 2. Understorey consisted mainly of bracken accompanied with seedlings and coppices at Messmate 1 and grass at Messmate 2. In contrast, Mountain Ash is an obligate seeder and killed by wild fire, regenerating via seed germination. Seedlings appeared as soon as November 2009 at the Mountain Ash site and grew to about a metre tall by February 2010 and about 2 metres by December 2010. Because of the intensity and extent of the fire (272 000 ha), no un-burnt sites could be found in the vicinity of the study site.

The moss patches at Messmate 1 and Mountain Ash sites were mapped in February 2010. For each 1m<sup>2</sup> square, the moss was recorded as none, less than 50%, 50-75%, 75-100% or 100% cover. The moss beds covered 66% of the Messmate 1 site and 74% of the Mountain Ash site (see Figure 4.1). Expansion of the moss beds was slow at the Messmate 1 site whereas hardly any bare patch of soil was left by July 2010 under the Mountain Ash.

Collars for measuring soil respiration were installed on 16 October 2009. The collars were 200 mm diameter and 50 mm tall. They were inserted into the soil to a depth of about 20 mm and maintained in place with alloy pegs. At both the Messmate 1 and Mountain Ash sites, five sets of two collars were installed. For each set, one collar was on soil covered by moss and the other one was nearby on a patch of bare soil, from here on respectively called Moss plots and No Moss plots. Collars in each set sat at less than 1.5 m from each other. Nine single collars were installed at Messmate 2 (No Moss).

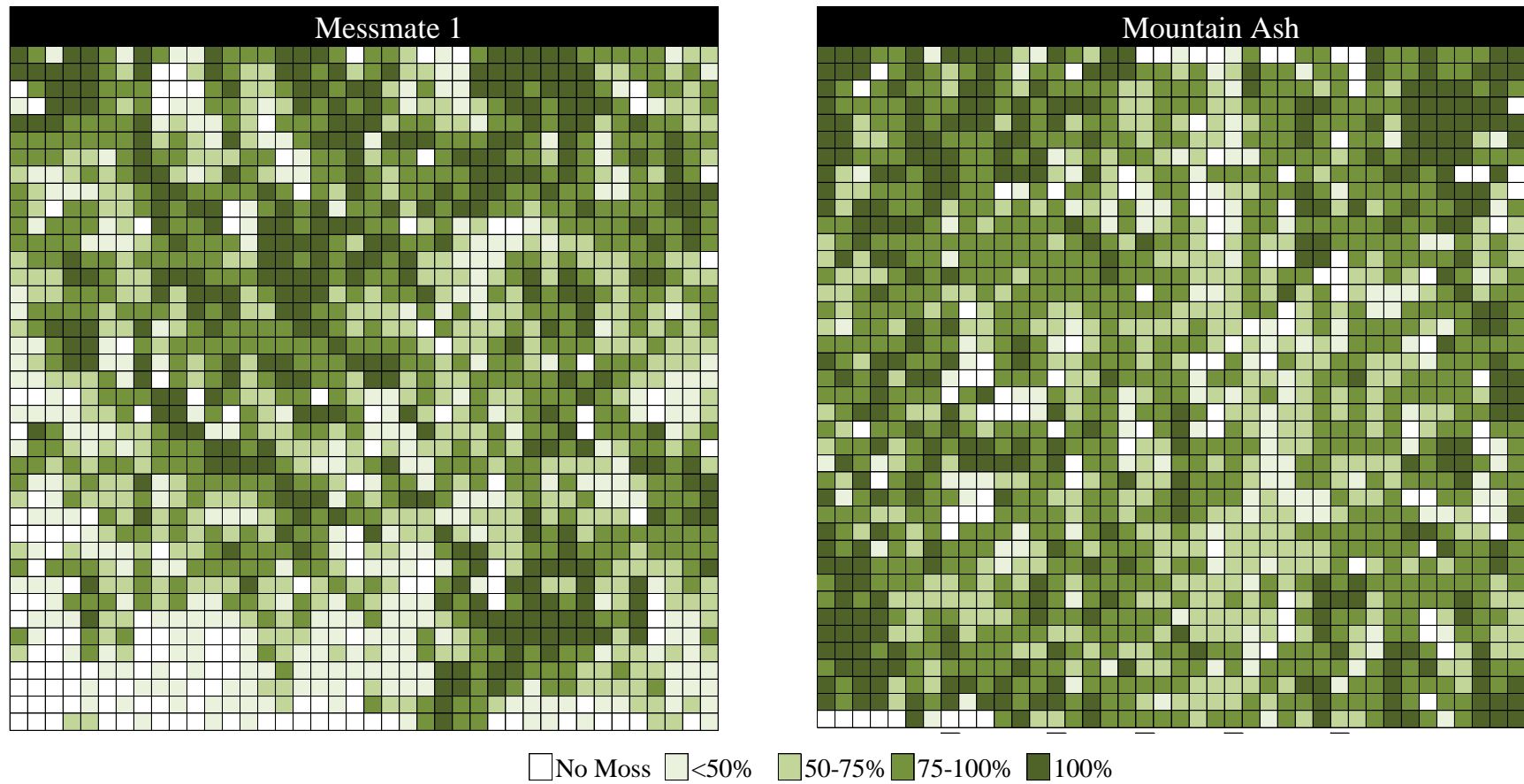


Figure 4.1.

Mapping of the moss beds at the Messmate 1 and Mountain Ash sites of the Mt Disappointment State forest.

By February 2010, the growth of the Mountain Ash seedlings at the Mountain Ash site was rendering impossible the use of the 200 mm diameter collars. Therefore, in March 2010, the experimental design was adapted. The size of the collars was reduced to 100 mm diameter. They were installed between seedlings in a way that excluded seedlings from the inside of the collars. It thus avoided both interference between above ground respiration and soil respiration and disturbance of the microenvironment around the collars that would have resulted from pruning the seedlings. Soil respiration was monitored for two months using both collar sizes in order to ensure that there was no difference between the two. The results were near identical differing by no more than 7% on average (t test,  $P > 0.05$ ).

Soil respiration was measured at monthly intervals starting in November 2009, one month after the collars were installed, until March 2011 (17 months). Measurements of soil respiration were made using Vaisala carbocap GMP343, an infra red gas analyser, mounted on a 5.25 litre lid (1.63 litre lid after March 2010 at the Mountain Ash site) and linked to a Vaisala MI70 data logger. At each site, the Vaisala was calibrated for atmospheric pressure and relative humidity measured with a Kestrel 3500 mini weather station. The lid was placed on the collar and the lid-Vaisala-collar association worked as a static chamber. The lid was left on the collars for 10 minutes. The recording during the first two minutes was discarded in order to allow the  $[CO_2]$  to stabilise. Respiration rates were calculated from the linear increase in  $[CO_2]$  recorded by the Vaisala for the remaining eight minutes. Soil temperature at 100 mm depth and soil volumetric water content were recorded beside the collars during the measurements with a MP406 probe linked to a MPM160 meter (ICT international, Armidale Australia).

Every three months a comprehensive soil sampling was conducted. At Mountain Ash and Messmate 1, two cores of soil were taken near the collars on both the Moss and No Moss patches, ie five soil samples of bare soil, five soil samples of moss-covered soil per plot. At Messmate 2, one sample was taken beside each collar and then pooled with the nearest two samples for a total of three bulked samples. Soils were sieved to 2 mm and analysed for gravimetric water content and pH each month as well as for microbial biomass on the more intensive samples collected quarterly. The “water drop method” (Watson and Letey, 1970) was used on the Messmate 1 soils sampled in February 2011 to investigate potential differences in hydrophobicity between Moss and No Moss soils. Triplicate of 1 g of each soil as well as the black layer that sits between the moss layer and the soil were oven dried at 65 °C for 48 hours and then cooled in a desiccator at ambient temperature for another 48 hours. Four drops of deionised water were placed on each soil. The time it took the soils to absorb the water was recorded.

#### *4.2.2 Snowy Plains*

The second experimental site was located in the Snowy Plains (36°06'05"S 148°31'35"E) of the Snowy Mountains in New South Wales, Australia at an altitude comprised between 1475 m and 1700 m above sea level. The mean annual minimum temperature at the study site is 1.7 °C and the maximum 10.2 °C. Average annual precipitation is ~ 1600 mm. Sites are snow covered for approximately three months of the year from July to September. Soils are derived from the Silurian Granodiorite, approximately 433 ±1.5 million years.

Three woodland sites were established on private property used in the summer time for cattle grazing. The sites were sub-alpine woodland of *Eucalyptus pauciflora* (Snow Gum) with an understorey dominated by woody shrubs such as *Bossiaea foliosa* (N-fixing), *Leucopogon* spp. (Ericaceous) and *Tasmannia xerophila* or a herbaceous understorey dominated by grasses such as *Poa* spp. *Eucalyptus pauciflora* regenerate from fire by both shoot sprouting or coppicing and seed germination. In this study, I considered only ungrazed plots that were fenced to exclude cattle but not native animals.

Each plot was 80 m x 40 m. Plots were established in the 2006/2007 Australian summer. A subplot covering one half of each plot was burnt in early spring in March 2007. The fire recreated the intensity of a wildfire. It incinerated the understorey and litter layer and killed some of the trees. The biomass of the living trees in the unburnt woodland (180 tonnes ha<sup>-1</sup>) was twice as much as the tree biomass in the burnt woodlands (85 tonnes ha<sup>-1</sup>).

In June 2009, collars (200 mm diameter x 50 mm tall) were installed for measuring soil respiration. Five collars were inserted 20 mm into the soil of each burnt and unburnt woodland plot.

Soil respiration measurements started on 23 October 2009, more than four months after installation of the collars, and lasted until 27 May 2010. A second campaign

went on after winter from October 2010 and up to March 2011. Soil respiration was measured using the same method as the one used at Mount Disappointment.

Soil samples were taken on the day of soil respiration measurement. For each sites-treatment combination, four cores were bulked together and sieved to 2 mm. Soil moisture analysis and pH measurements were performed on each type of soils. Microbial biomass count was performed on the samples taken on the first and last measurement days.

#### *4.2.3 Statistical analysis*

All analysis was performed in R. Repeated measure ANOVA (RM ANOVA) with treatment and date as factors was used to detect any effect of fire on soil respiration in the woodlands of the Snowy Plains for the whole length of the experiment. It was also used for test for variation between Moss and No Moss plots at Messmate 1 and the Mountain Ash site across time. Student t-test was used to test for differences in respiration rates each month between Moss and No Moss plots at Mountain Ash site and Messmate 1 and burnt and unburnt woodlands of the Snowy Plains. The effect of soil temperature and soil moisture on soil respiration at all sites was calculated using linear regression.

Two-way ANOVA was also used for differences in soil water content and soil temperatures between burnt treatments in the Snowy Plains and Moss and No Moss plots at Messmate 1.



### **4.3 Results**

#### *4.3.1 Belowground respiration*

Soil respiration rates at Mt Disappointment could be separated into two distinct periods: the first 10 months of measurements until September 2010, and then from this date to March 2011 (Figure 4.2). During the first period, soil respiration was stable, displaying very little seasonal variation during the first year of measurements at Messmate 1 and 2. There was however a surge in respiration in February 2010 at Messmate 1 and 2 and from January to March 2010 at the Mountain Ash site. Rates in February were twice as fast as rates in January. On the other hand, from September 2010, soil respiration increased each month following the seasonal pattern that is usually observed in soil respiration. Rates were greater during the 2010/2011 season than the previous year at Messmate 1 and 2 but to a lesser proportion at the Mountain Ash site. In any case soil respiration rates followed a similar pattern along the year at all sites albeit with different values. Across time, the Mountain Ash site showed greater extremes in respiration rates than did the two other sites. In contrast, it did not show a strong seasonal effect similar to the one observed from September 2010 until March 2011 at Messmate 1 and 2, especially if discounting the peak of respiration in January 2011 under the Mountain Ash forest.

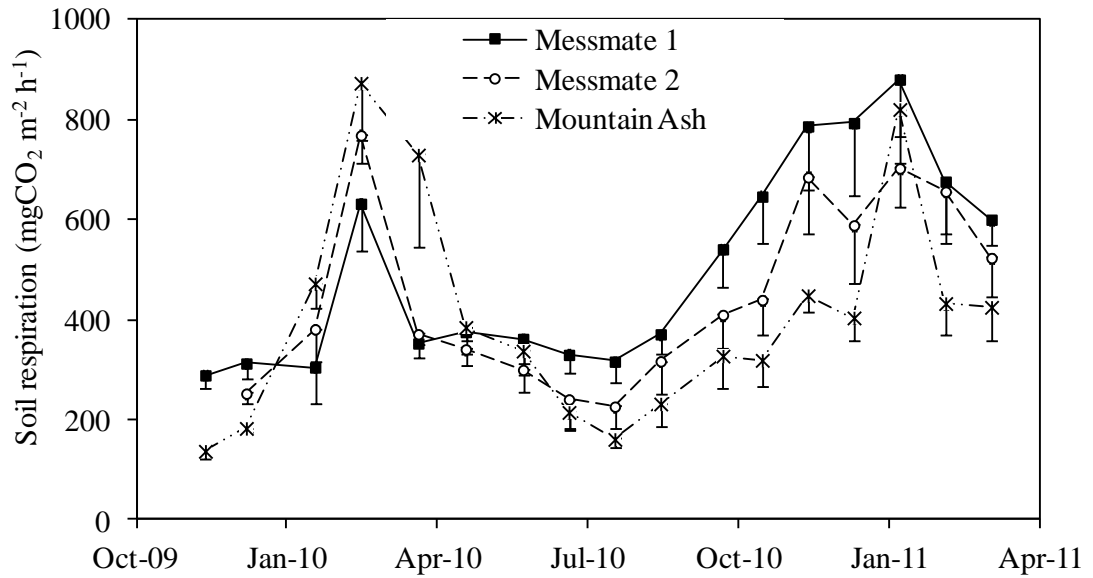


Figure 4.2.

Rates of soil respiration at the three sites of the Mount Disappointment State forest

For each measurement, rates of soil respiration were significantly greater from moss-covered soils than bare soil at the Messmate 1 site (Figure 4.3, *t* test,  $P < 0.05$ ). However, differences at the Mountain Ash site between moss-covered and bare soils were not significant (ANOVA,  $P < 0.05$ , data not shown). For this reason, measurements from collars on bare soil at this site were not taken after November 2010. Rates at the Messmate 2 site were in the mid-range of those recorded at the two other sites.

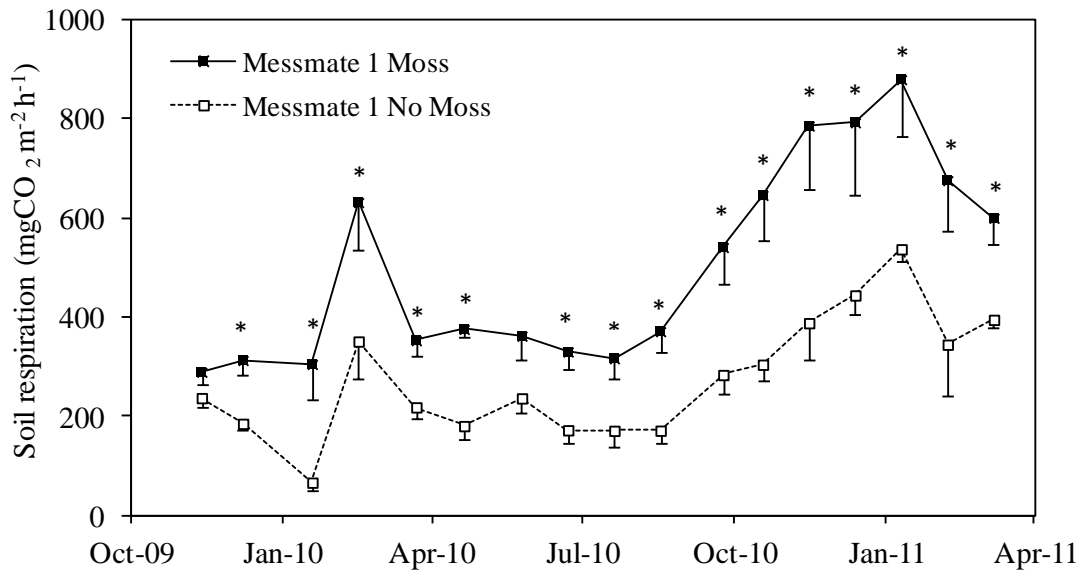


Figure 4.3.

Rates of soil respiration at the Messmate 1 site of the Mt Disappointment state forest. \* indicates significant difference (t test,  $P < 0.05$ )

Rates of belowground respiration at the Snowy Plains were variable from month to month and were greater during the second, wetter year of measurements (Figure 4.4). For example, soils of the unburnt woodlands displayed a seasonal maximum respiration rate of  $945 \text{ mgCO}_2 \text{ m}^{-2} \text{ h}^{-1}$  in January 2011, compared to a peak at  $544 \text{ mgCO}_2 \text{ m}^{-2} \text{ h}^{-1}$  in February 2010 during the previous summer. There was a clear seasonal effect in both years although it was somewhat subdued in the summer 2009-2010 due to a drop in respiration for the month of January 2010. Minimum temperatures on the day of measurement were a low  $3.7 \text{ }^\circ\text{C}$ , in contrast to the monthly mean of  $12 \text{ }^\circ\text{C}$ .

No significant effect of fire was recorded (RM ANOVA,  $P < 0.05$ ), despite the burnt woodlands presenting a different aspect to unburnt woodlands. The unburnt woodlands can be classified as Snow Gum woodland with shrubby understorey

whereas the burnt woodlands are Snow Gum woodlands with grassy understorey due to the shrubs being replaced with grass. Averaged over the three plots, live tree biomass of the unburnt woodlands was more than double that of the burnt woodlands.

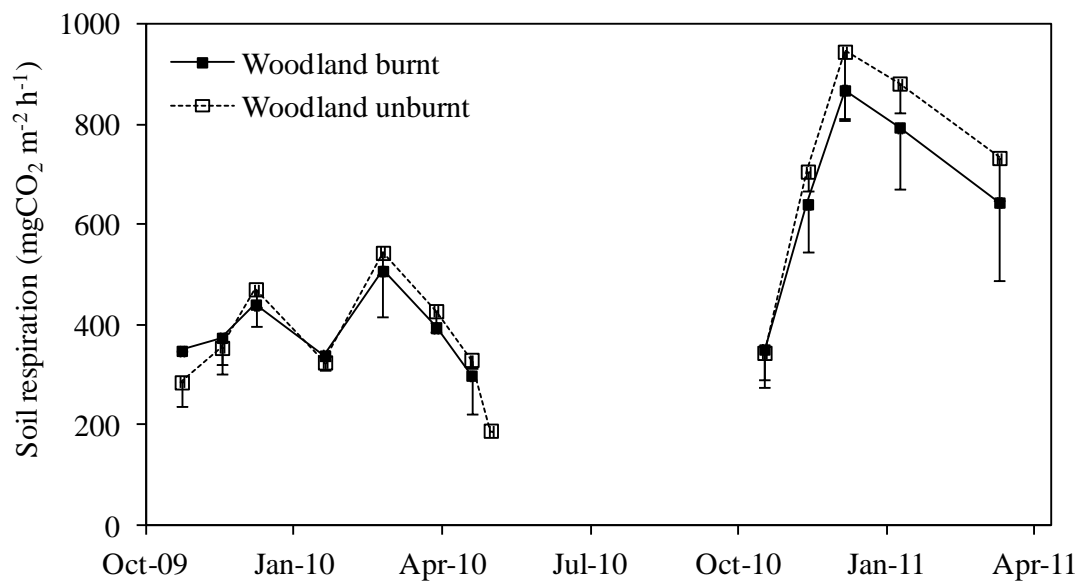


Figure 4.4.

Rates of soil respiration at the Snowy Plains. Rates are averaged over the three woodland sites.

#### 4.3.2 Effect of soil moisture

Soil moisture varied greatly over time at both Mt Disappointment and Snowy Plains (Figure 4.5). Soil moisture was greater during the second half of 2010 and early 2011 at both sites. At Mount Disappointment, soil water content was stable between November 2009 and April 2010, except for a peak in February 2010. Soil water content increased significantly in the winter months of 2010 and remained elevated until early summer. Soil moisture was significantly different and usually greater in the moss-covered plots at Messmate 1 than the No Moss plots (Two-way ANOVA,

$P < 0.05$ ). The black layer between moss beds and the soil was very hydrophilic whereas the moss-covered and bare soils did not present any difference in hydrophobicity. The black layer absorbed the four drops of water in less than three seconds whereas it took an average of ten seconds for the soils to do so.

At the Snowy Plains, soil moisture was variable in the 2009-2010 season but greater and more stable during the second season of measurements, reflecting rainfall at the sites. There was no difference in soil moisture between the burnt and unburnt plots (Two-way ANOVA,  $P > 0.05$ ).

Soil respiration was positively linked with volumetric water content at Messmate 1 for both the moss-covered and bare soils and in the Snowy Plains (linear regression,  $P < 0.05$ ). No such link could be established at Messmate 2 and under the Mountain Ash trees at Mt Disappointment.

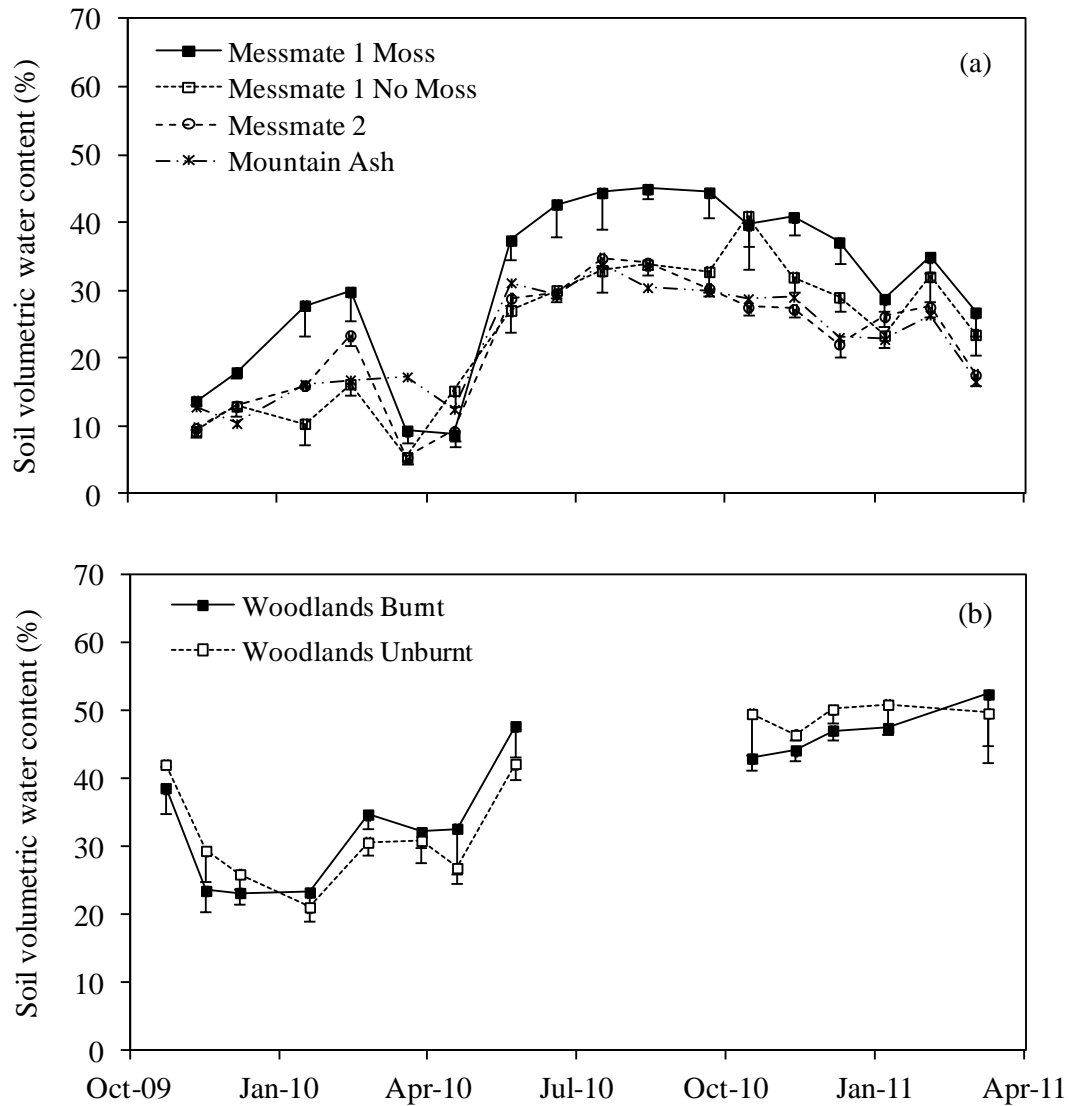


Figure 4.5.

Volumetric water content at all sites of Mt Disappointment (a) and the Snowy Plains (b)

#### 4.3.3 Soil temperature effect

Soil temperature was a major governor of soil respiration at the Messmate 2 and the Mountain Ash sites of Mt Disappointment and at the Snowy Plains (linear regression,  $P < 0.05$ ). However, no relation was established at Messmate 1 (linear regression,  $P > 0.05$ ). In the Snowy Plains, soil temperatures were overall significantly greater in unburnt than in burnt woodlands (RM ANOVA,  $P < 0.05$ ).

There was a very significant positive impact of the combination of soil moisture and soil temperature on belowground respiration for Messmate 1 for both moss-covered and bare soils as well as at Messmate 2 at Mount Disappointment and in the Snowy Plains (general linear model,  $P < 0.01$ , Table 4.1).

Table 4.1.

Coefficients of equations generated by general linear model of soil respiration as a function of soil volumetric water content and temperatures at Mt Disappointment and the Snowy Plains. All models are significant at  $P < 0.01$

	Volumetric Water Content	Temperature	Intercept	R square
Messmate1 Moss	12.0	36.5	-393.9	0.264
Messmate 1 No moss	4.5	11.2	-31.4	0.109
Messmate 2	10.9	26.2	-272.7	0.284
Woodland Burnt	10.3	33.5	-393.8	0.348
Woodland Unburnt	9.7	40.7	-387.2	0.531

#### 4.3.4 Cumulative soil respiration

Patterns of cumulative soil respiration were similar at Messmate 2 site and both the Moss and No Moss plots at the Messmate 1 site albeit with a steeper slope for the former two (Figure 4.6a). Cumulative respiration of the Mountain Ash forest was more variable reflecting the extremes in instantaneous rates of belowground respiration we observed at this site. Between December 2009 and October 2010, more CO<sub>2</sub> was released from the soils of the Mountain Ash forest than from the soils of the other sites. There was a shift from October 2010 when the cumulative soil respiration of the Mountain Ash site decreased to values lower than at Messmate 2 and the Moss plots of Messmate 1 sites.

Cumulative respiration at the Snowy Plains revealed a much steeper slope on the second year the site was visited (Figure 4.6b). Soils of burnt and unburnt woodlands released similar amount of CO<sub>2</sub> in the atmosphere during the time that the sites were monitored.

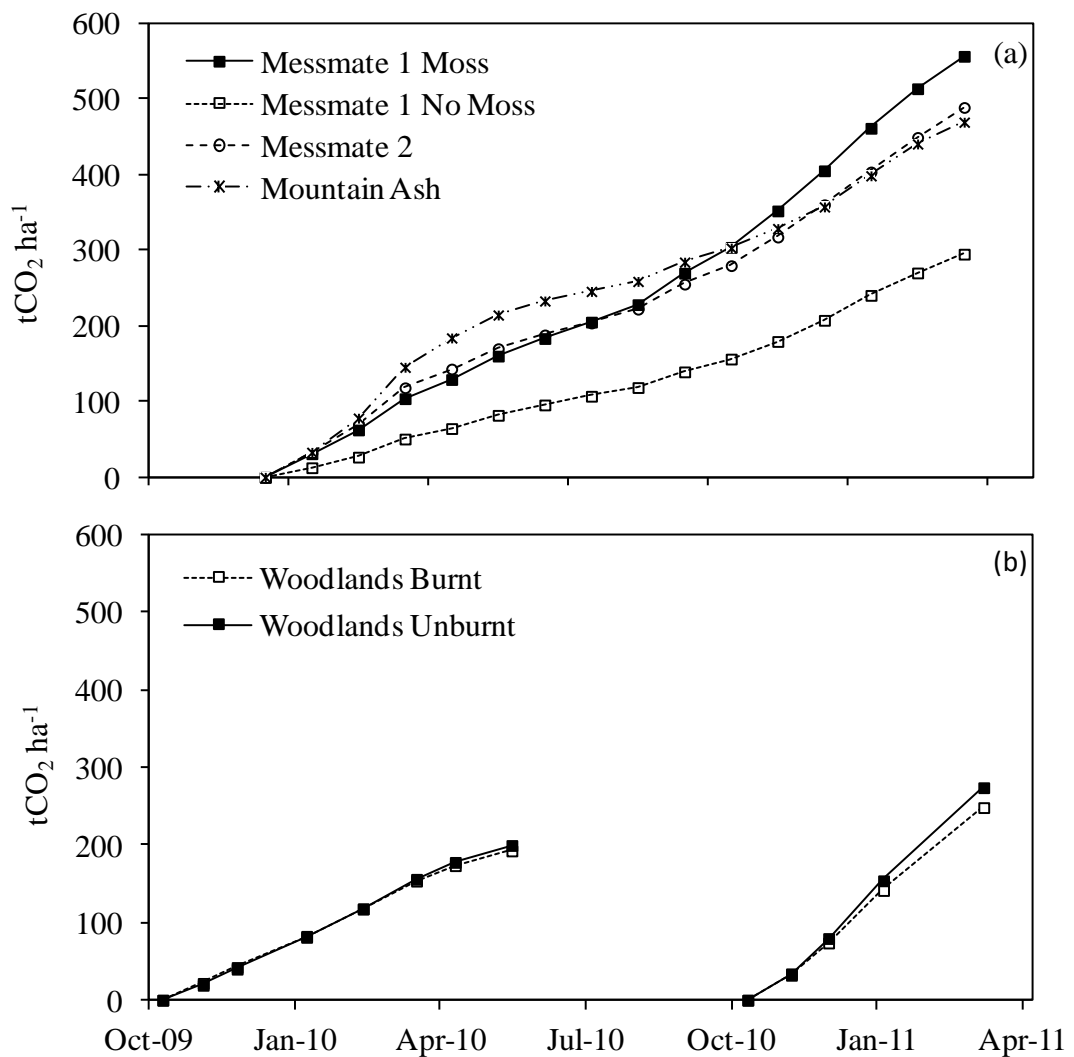


Figure 4.6.

Cumulative soil respiration at the three sites of the Mount Disappointment state forest (a) and the Snowy Plains (b)



#### *4.3.5 Weather*

The year 2010 was the fifth wettest year on record in the State of Victoria and the wettest year since 1974. It was the third wettest year on record in New South Wales and the wettest since 1956. Most of the rain fell in the second half of the year. The station of Kilmore Gap, the closest to the Mount Disappointment sites recorded 1088.2 mm of rain in 2010, 160% of the average annual rainfall for the location. The closest weather station of the Snowy Plain recorded 1447 mm of rain for the same year, representing 130% of the annual average. Maximum temperatures were close to the annual average but minimum temperatures were warmer by half a degree than the annual average in both Victoria (Stern et al. 2011) and NSW (Bureau of Meteorology).

#### *4.3.6 Microbial biomass*

Except for August 2010, microbial biomass was consistently greater in the bare soil than the moss-covered soil of Messmate 1 by 11% to 100% (Figure 4.7). The amount of microbial biomass was variable with time on all sites but overall the soils covered with Mountain Ash had greater values than the soils of Messmate 1 and 2. An increase in microbial biomass in February 2010 corresponding to a peak of respiration and only a few days after the sites had received more than 50mm of rain in 48 hours.

Microbial biomass presented little variation between treatments at the Snowy Plains (ANOVA,  $P > 0.05$ ).

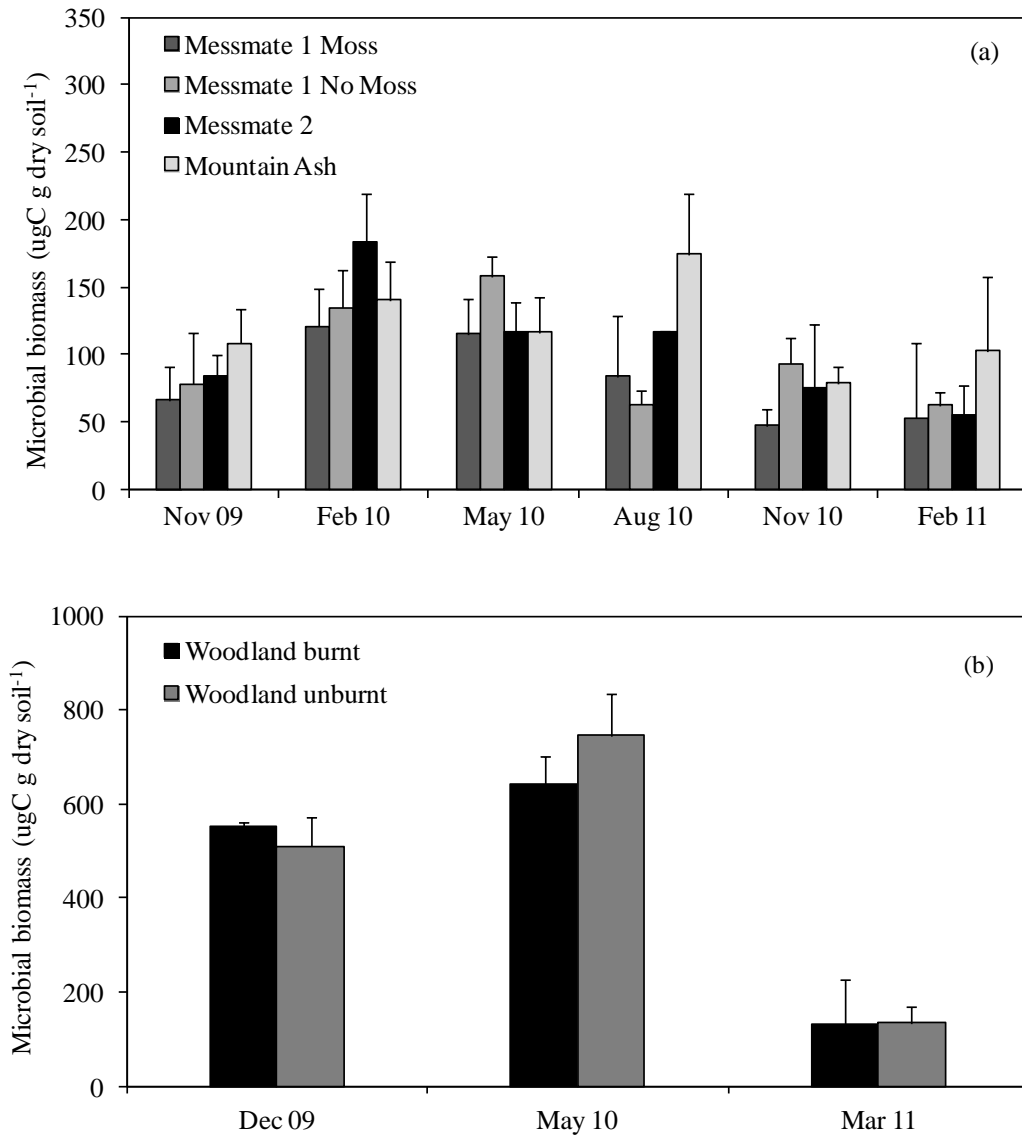


Figure 4.7.

Soil microbial biomass from chloroform fumigation and 0.5M K<sub>2</sub>SO<sub>4</sub> extraction at (a) Mt Disappointment and (b) the Snowy Plains

#### ***4.4 Discussion***

Soils under moss beds of the Messmate 1 site produced more CO<sub>2</sub> than the contiguous bare soils. The proximity of the Moss and No Moss collars strongly suggests the difference is not due to tree roots. The moss beds were very distinct at the time collars were installed. As time passed, the moss also colonised bare soil patches. However, respiration rates of Moss plots remained greater than rates recorded to No Moss plots. This result supports the original hypothesis that moss beds are an indicator of soil processes. The original area covered by moss had a strongly hydrophilic black layer between the soil and the moss. This layer represents an accumulation of soil and charred litter that was washed and settled in depressions or in areas presenting a greater surface tension between soil and particles. DeBano (2000) explained that soil water repellency post-fire depends on fire intensity and behavior and soil characteristics (ie texture, water content, type and quantity of SOM). In the case of mid intensity fires (ie soil temperature between 175 to 200°C), the development of an underground repellent layer may lead to waterlogging of surface soils, whereas soil erosion might be favored by the disappearance of surface repellency. In contrast, high intensity fires with soil temperatures reaching between 250 and 300 °C tend to burn the repellent layer, reducing soil hydrophobicity (DeBano 2000). The black layer seemingly created a moist environment, rich in nutrients and favourable to the establishment of moss and microbes. In turn moss provided an insulating and shading layer, reducing evaporation and resulting in the consistently greater soil water content. Microbial biomass was generally greater in the No Moss plots than the Moss plots. Greater microbial activity and turnover rates, stimulated by the moist environment, may thus be causing greater rates of heterotrophic soil respiration from moss-covered soils. The mapping of the moss

patches at the Messmate 1 site showed that they covered about 66% of the total area of the site. Based on the calculation of cumulative soil respiration, moss-covered soils contributed approximately three times more than bare soils to the respired soil CO<sub>2</sub> at Messmate 1 over the course of this study (367 tCO<sub>2</sub> ha<sup>-1</sup> versus 130 tCO<sub>2</sub> ha<sup>-1</sup>).

The results of the present study indicate that, when considering belowground respiration, soils of forest ecosystems dominated by resprouting trees (Messmate 1 and 2 and Snowy Plains) are more resilient than those dominated by obligate seeders (Mountain Ash). The return of a normal seasonal pattern of soil respiration at Messmate 1 and 2 from September 2010 and the absence of strong reaction to extreme climatic events suggest that these two sites were on path to recovery, 18 months after the fire storm. Similarly, two and a half years after the burn, burnt and unburnt plots at the Snowy Plains did not show any significant difference in soil respiration, despite fire having reduced living tree biomass by half and grass replacing shrubs as the understorey on the burnt plots. In contrast, two years after the fire, the Mountain Ash sites were still subject to sudden increases and decreases in soil respiration rates following rainfall events and/or extremes in temperatures, such as those observed in January 2011.

The surge in soil respiration rates in February 2010 illustrates the extreme sensitivity of soil respiration to the vagaries of the weather after removal of vegetation by fire. Soil water content and soil temperature are the main drivers of belowground respiration. When soil temperatures are warm to hot (>16-20°C, Almagro et al.

2009), belowground respiration is directly dependant of soil moisture. There was a positive effect of the combination of soil moisture and soil temperatures on soil respiration at all sites in Mount Disappointment. A surge in respiration rates in February 2010 could be expected due to unseasonably high level of soil moisture combined with hot soil temperatures characteristic to this summer month (18.8 to 22.3°C across sites). The extent of this surge (eg a doubling of respiration rates from the previous month) and the time lag after the precipitation event (eg three days) is less common in normal conditions. Soil moisture was exceptionally high for two reasons: First, four and three days before measurements, the region received respectively 35.8 mm and 19.8 mm of rain, accounting for more than 66% of the total amount of rain since the January measurement. Secondly, there was a low level of evapotranspiration due to the lack of canopy cover at both the tree level and understorey level. Such an increase in soil moisture following the removal of canopy cover by fire has been observed in forests such as Oak Pines and Ponderosa Pines (Dumas et al. 2007, Hamman et al. 2007). Soil temperatures might also have been increased from their pre-fire values by reduction of the canopy, understorey and litter layer that provide shading and insulation to soils (Czimczik et al. 2006, Dumas et al. 2007). It can take less than two years to observe a return of pre-fire soil temperatures in burnt sites (O'donnell et al. 2009) whilst non-functioning understory and trees would have transpiration greatly reduced thus increasing soil moisture (Dumas et al. 2007, Hamman et al. 2007). A contiguous trenching experiment at the Mountain Ash site (see Chapter 5) has revealed that the removal of seedlings led to a significant increase of soil temperatures in the trenched plots and a general increase of soil moisture. Because of the fire, the tampering effect of the vegetative cover has disappeared, rendering soil respiration more responsive to extreme weather events.

As a consequence, the impact of weather on soil respiration will diminish earlier in plant communities that are more resilient to fire as was illustrated by the doubling of the soil respiration rates under Mountain Ash stands in January 2011 whilst rates hardly increased at the two other sites of the Mt Disappointment forest.

At the Snowy Plains, respiration rates of burnt and unburnt woodlands were similar. Microbial biomass and total tree biomass were also similar in between plots, but the living tree biomass was twice as much in the unburnt plots than in the burnt plots. At these sites, heterotrophic respiration of woodland with shrubby understorey, characteristic of the unburnt sites, is greater than respiration in woodland with grassy understorey, characteristic of the burnt sites (Jenkins and Adams 2011). Autotrophic respiration must thus be greater on the burnt plots than the unburnt plots to compensate for the deficit in heterotrophic respiration. The grassy understorey might be a major contributor of the autotrophic component of soil respiration in the burnt plot. In addition, the trees surviving the fire may have benefited from the opening in the canopy and the reduction in competition for nutrients from the dead trees in order to increase their rates of photosynthesis. This could translate into greater specific root respiration from the living trees since rhizosphere respiration is linked directly to the production of photosynthates (Hogberg et al. 2001).

The irregularity of respiration rates at more disturbed sites such as the Mountain Ash site translates into variation in cumulative soil respiration. Mountain Ash stands were clearly the biggest contributors to soil respired CO<sub>2</sub> at the Mt Disappointment forest from the start of measurements in November 2009 until October 2010. At this date,

the cumulative amount of CO<sub>2</sub> respired by the Mountain Ash soils dropped below that respired at Messmate 1 (Moss) and two months later Messmate 2. Again the soils of the Snowy Plain did not show any differences between treatments including on the second campaign of measurements despite wetter weather and wetter soils. This confirms that three and four years after fire, soil microbial community and aboveground vegetation have recovered in a way that soil respiration is not affected by the burn anymore.

#### ***4.5 Conclusion***

Recovery of soil respiration to pre-fire pattern was quicker on sites dominated by resprouting trees than sites covered with seeder trees. However, spatial variation of respiration rates within site persisted even after the seasonal pattern of respiration had returned. Future studies on the impact of fire on belowground respiration will have to consider the potential for spatial variation in respiration rate. Early visits of burnt sites following fire are required in order to identify any clues that the sites may present a mosaic of areas with different respiration rates such as the one we observed at the Messmate 1 site at Mt Disappointment. As fire may become more common in regions already subjected to regular burning, future research should focus on the role of the increase in frequency of fires. Fire may also become a regular feature in regions where it is absent or only occurs exceptionally; the response of the belowground respiration of these ecosystems will also need to be studied in order to establish accurate regional and global carbon budgets.

## *Chapter 5*

### *Contributions of tree roots to soil respiration: field and laboratory studies*

#### *5.1 Introduction*

Soil respiration is usually described as being the sum of heterotrophic respiration, originating from free living fungi and bacteria decomposing soil organic matter (SOM), and autotrophic or rhizosphere respiration from roots and associated mycorrhizal fungi feeding on plant photosynthates. Each of these components is often studied as separate entities (Hanson et al. 2000, Kuzyakov and Larionova 2005).

One of the common techniques used to partition total soil respiration is to isolate sub plots of soils by trenching accompanied by removal of aboveground vegetation (Hanson et al. 2000, Kuzyakov 2006). The resulting CO<sub>2</sub> efflux from trenched plots is then considered to be solely of heterotrophic origins, and can thence be used to partition respiration (autotrophic and heterotrophic) from nearby un-trenched plots. Although widely used, trenching presents well-known limitations: disturbance effects of trenching include changes in soil water content and soil temperature, interference of root decomposition fuelling microbial respiration (Diaz-Pines et al. 2010) and increases in the upward flux of CO<sub>2</sub> due to the absence of root respiration (Jassal and Black 2006). All these factors can lead to overestimation of heterotrophic respiration. Recent studies have shown that total belowground respiration is more complex than just the sum of its component parts, due especially to priming effects in the



rhizosphere (Cheng and Kuzyakov 2005, Dijkstra and Cheng 2007a, Zhu and Cheng 2011). Rhizosphere priming effects (RPE) include impacts by roots and associated fungi on the decomposition of soil organic carbon by heterotrophs. RPE can be both negative and positive - from a 50% reduction to a 320% increase in heterotrophic respiration (Cheng and Kuzyakov 2005). The extent of priming effects are governed by plant species (Dijkstra and Cheng 2007a) and phenology (Cheng et al. 2003), atmospheric concentration in CO<sub>2</sub> (Cardon 1996), plant biomass (Dijkstra et al. 2006), nutrient status of soil (Liljeroth et al. 1994) and soil moisture (Dijkstra and Cheng 2007b). Many of these are in turn predicted to vary under climate change scenarios. Beside the well-known increase in atmospheric [CO<sub>2</sub>], climate change models are forecasting shifts in species composition, extension of the growing season, an increase in plant biomass triggered by the fertilising effect of elevated [CO<sub>2</sub>] and increases in atmospheric and soil temperatures (Solomon et al. 2007). RPE have not been widely studied and usually involve crops or non-woody plants except for a few cases (Bader and Cheng 2007, Cheng 2009, Cheng et al. 2003, Dijkstra and Cheng 2007a, Dijkstra et al. 2006, Zhu and Cheng 2011). Studies conducted in the laboratory involve a high level of soil disturbance which can mask or increase the impact of rhizosphere priming effect on SOM decomposition, particularly if experiments are of short duration (Bader and Cheng 2007, Dijkstra and Cheng 2007a, Zhu and Cheng 2011).

Because of the lack of knowledge of specific mechanisms of RPE, soil respiration and climate models still tend to consider heterotrophic and autotrophic components of belowground respiration separately, rather than as part of a continuum between

plants and soil. Hence it can be assumed that the accuracy of these models could be enhanced by improving the understanding of this crucial aspect of soil respiration.

Here I used a field technique to separate components of soil respiration as well as a novel, flow-through, laboratory based gas monitoring system measuring heterotrophic respiration only (respirometer), in order to isolate autotrophic and heterotrophic parts of soil respiration and evaluate possible rhizosphere priming effects.

## ***5.2 Materials and Method***

### *5.2.1 Site description*

The experimental site is located in the Mount Disappointment state forest about 50 km North of Melbourne, Australia at an altitude of around 800m above sea level. See Chapter 4 for details. The Mountain Ash (*Eucalyptus regnans*) site was chosen for this study. The study plot is a 40 m by 40 m square. A detailed description of the soils is given in Adams (1984). In brief, the soil is a brown earth (Udic Ustochrept) with an average pH of 5.7 (See Table 5.1), total carbon content in surface 5 cm of 10.1% and total N of 0.47%. Atmospheric [CO<sub>2</sub>] at this site, as measured between November 2009 and March 2011, was 375 ppm.

The Mountain Ash site was amongst the 330,000 ha of land burnt by the “black Saturday” bushfires, an extreme fire event, on 7 February 2009. The high intensity wildfire totally defoliated the crown of the trees and incinerated the litter layer and

understorey. As Mountain Ash are obligate seeders, the trees were killed by the fire, the stand regenerated from seeds. The first seedlings appeared in October 2009, about seven months after the fire storm.

### *5.2.2 Trenching and soil respiration measurements*

In March 2010, collars of 100 mm diameter and 80 mm tall were installed for measuring soil respiration. The collars were inserted into the soil to a depth of about 20mm and maintained in place with alloy pegs. They were installed between seedlings in a way that excluded seedlings from the inside of the collars and thus avoided interference between above-ground respiration and soil respiration, while not hindering seedling growth. Measurements were taken from five collars and averaged to determine the rate of belowground respiration at the site. At the same time, five quadrats 1 m x 1 m were cleared by pruning the seedlings at their base. A trench was dug around each quadrat to a depth of 40 cm, about 20 cm below the root zone, and plastic barriers were installed before refilling the gap between soil and barrier. Each quadrat contained one collar therefore giving five trenched and five un-trenched collars from which respiration was measured. Any plants that appeared inside the trenched plots were removed. The pruned seedlings were kept, dried at 60°C and weighed to an average of 340 g per plot (ie 3.4 t ha<sup>-1</sup>). In March 2011, the average biomass of the seedlings at the site had increase to an average of 786 g m<sup>-2</sup> (ie 7.86 t ha<sup>-1</sup>). The March 2010 sampling revealed that the number of stems totalled nearly 700,000 per hectare.

Soil respiration was measured at monthly intervals starting in April 2010 at the trenched plots, one month after the collar installation. Measurements of soil respiration were made using a Vaisala carbocap GMP343 mounted on a 1.63 l lid and linked to a Vaisala MI70 data logger. The lid is placed on the collar and the lid-Vaisala-collar association works as a static chamber. The lid was left on the collars for 10 minutes. The recording during the first two minutes was discarded in order to allow the [CO<sub>2</sub>] under the lid to stabilise. The rate of CO<sub>2</sub> efflux was calculated from the slope of the evolution of [CO<sub>2</sub>] during the remaining 8 minutes. Soil temperature at 100mm depth and soil volumetric water content were recorded beside the collars during the measurements.

Soil was collected from the un-trenched plots once a month by taking four 100 mm deep soil cores and pooling them. Every three months and on the last visit in March 2011, a more intensive sampling was made by taking and pooling two cores of soil adjacent to each collar. One core was collected from each trenched plot and kept separate. Soils were sieved to 2 mm and analysed for gravimetric water content and pH each month as well as for microbial biomass on the more extensive samples collected quarterly.

In parallel to field measurements, I completed laboratory measurements of heterotrophic respiration from the soil samples collected quarterly in August 2010, November 2010, February 2011 as well as March 2011. The respirometer technique described in Jenkins and Adams (2010) was used: 80 to 100 g of fresh soil, sieved to 2 mm, was added to polycarbonate cylinder connected to an infra-red gas analyser (IRGA) and differential oxygen analyser (DOX) for CO<sub>2</sub> and O<sub>2</sub> analysis. The tubes

were let to equilibrate at constant temperatures in a water bath for an hour prior to and during measurements. I measured respiration at a range of temperatures 5-10-15-20-25°C corresponding to the soil temperatures observed in the field between November 2009 and March 2011. Measurement started at the temperature closest to the soil temperature recorded in the field when collecting the samples. Respiration rates were calculated over 60 seconds after CO<sub>2</sub> concentration had remained steady for 3 minutes.

The respiratory quotient (RQ) of each soil was calculated as the ratio between effluxes of CO<sub>2</sub> and uptake of O<sub>2</sub>.

Table 5.1.

Soil characteristics at all soil sampling dates. Mean calculated from five samples from each of the un-trenched (U) and trenched (T) plots. Microbial biomass and labile carbon are given with standard error. Both soil temperature (Temp) and gravimetric water content (GWC) were measured in the field when sampling.

		Temp (°C)	GWC (%)	pH	Microbial biomass (µg C g soil <sup>-1</sup> )	Labile C (µg C g soil <sup>-1</sup> )
Aug-10	U	7.3	48.2	5.6	175 (35)	122 (27)
	T	8.9	46.6	5.5	134 (48)	107 (19)
Nov-10	U	16.4	41.9	5.8	79 (09)	133 (16)
	T	16.8	44.2	5.8	56 (27)	136 (10)
Feb-11	U	15.8	42.8	5.5	103 (42)	148 (41)
	T	20.2	43.2	5.7	76 (10)	117 (19)
Mar-11	U	16.6	33.8	5.5	94 (06)	44 (15)
	T	22.7	41.5	5.4	56 (11)	42 (13)

### 3.2.3 Statistical analysis

All statistical analyses were performed in R. Rates of soil respiration measured in the field from trenched and un-trenched plots were compared with a two sample t-test assuming equal variance. Linear regressions were performed to estimate the impact of soil volumetric water content and soil temperature on soil respiration for both treatments. Repeated measure ANOVA was used to compare respiration rates measured in the laboratory on soil from un-trenched and trenched plots.

### 5.3 Results

#### 5.3.1 Field experiment

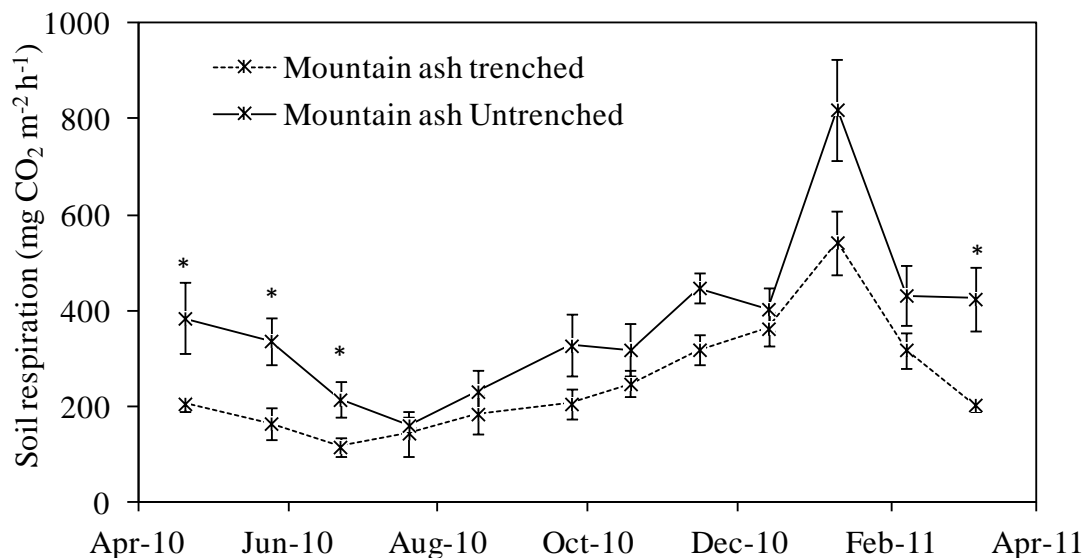


Figure 5.1.

Rates of belowground respiration measured *in situ* for the trenched and un-trenched plots of the Mountain Ash site of the Mt Disappointment state forest. Star symbol indicates significant difference in respiration rates (t-test,  $P < 0.05$ ).

*In situ*, the trenched plots showed slower rates of soil respiration compared to un-trenched plots, within one month of installation (Figure 5.1). Overall the difference between trenched and un-trenched plots was significant (two-way ANOVA,  $P < 0.05$ ). On a monthly basis, respiration rates were significantly faster (t-test,  $P < 0.05$ ) for April to June 2010 and March 2011. For these months, rates of respiration for trenched plots were approximately half those from un-trenched plots (Figure 5.2). On all other dates, trenched plots released between 63% and 90% of the amount of CO<sub>2</sub> that was released from the un-trenched plots. From these results and assuming that the respiration rates measured on the trenched plots are of heterotrophic origins only, heterotrophic respiration varied from 48% to 90% of total soil respiration.

Soil temperatures were significantly warmer (ANOVA,  $P < 0.05$ ) by an average of 2.1°C in the trenched plots compared to the un-trenched plots. Rates of respiration were linked to soil gravimetric water content and soil temperature for trenched plots but only to soil temperature for un-trenched plots.

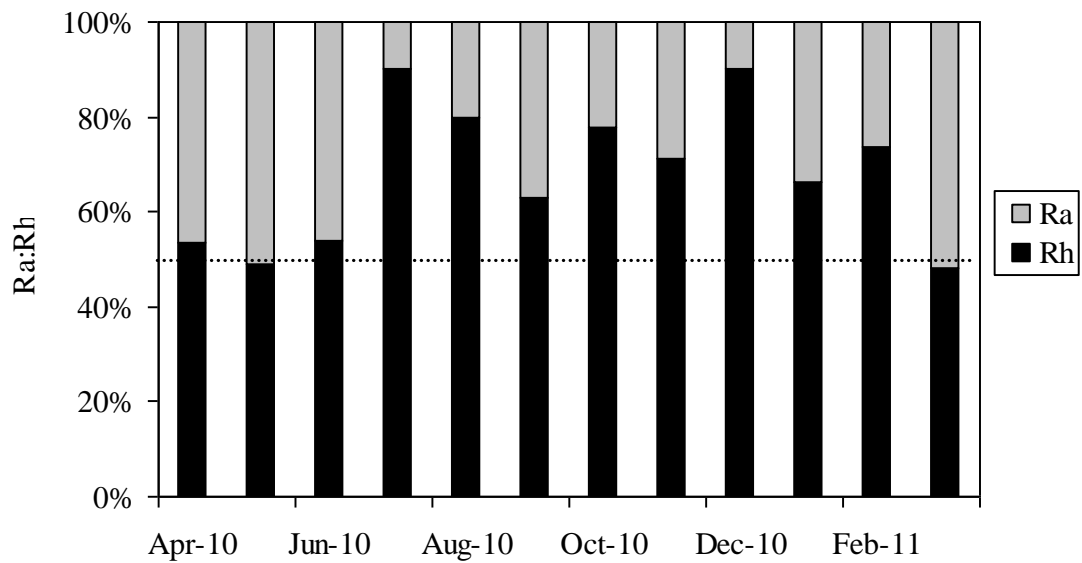


Figure 5.2.

Respective proportion of autotrophic (Ra) and heterotrophic (Rh) soil respiration as calculated from the mean of the five trenched plots and the mean of the five un-trenched plots



### 5.3.2 Laboratory experiment

Measured in the laboratory with the respirometer, heterotrophic respiration rates from soils of the un-trenched plots ( $R_u$ ) were consistently greater than those measured from the soils sampled from the trenched plots ( $R_t$ , Table 5.2). The ratio between respiration rates from the trenched and un-trenched plots ( $R_{u/t}$ ) was calculated from the rates of respiration recorded at temperature 15°C and above as follow:

$$R_{u/t} = \frac{R_u - R_t}{R_t}$$

It ranged from 9% to 162% depending on soil temperatures (between 15 and 25°C) and month of collection. Data from the lower temperatures (<15°C) were excluded as the relative standard errors at these temperatures averaged 45% and were up to 85%, much greater than for other soil temperatures (average of 23%).

There is a clear distinction between the first three series of measurements and the March 2011 measurements. For the August to February measurements,  $R_{u/t}$ , calculated from the mean of respiration rates between 15°C and 25°C, consistently averaged between 15% and 33%. In March 2011,  $R_{u/t}$  averaged 142% across temperatures (Table 5.2, Figure 5.3).

Table 5.2.

Rates of heterotrophic respiration ( $\mu\text{g CO}_2\text{-C g dry soil}^{-1} \text{ hour}^{-1}$ ) measured on the respirometer between 5°C and 25°C. Results with standard errors are averaged over measurements performed on five samples taken from the trenched plots ( $R_t$ ) and five samples taken from the un-trenched plots ( $R_u$ ).

Sampling date		5°C	10°C	15°C	20°C	25°C	Mean 15 to 20 °C
Aug-10	$R_u$	1.19 (0.45)	2.25 (0.40)	3.82 (0.50)	7.64 (1.17)	9.27 (1.34)	
	$R_t$	0.52 (0.17)	1.49 (0.20)	3.19 (0.56)	6.63 (1.14)	8.48 (1.24)	
	$R_{u/t}$	127%	51%	20%	15%	9%	15%
Nov-10	$R_u$	1.36 (0.46)	2.61 (0.55)	3.63 (0.25)	5.04 (0.25)	8.30 (0.42)	
	$R_t$	0.48 (0.10)	1.48 (0.24)	2.69 (0.34)	4.05 (0.43)	5.92 (0.50)	
	$R_{u/t}$	83%	77%	35%	25%	40%	33%
Feb-11	$R_u$	0.26 (0.04)	1.34 (0.15)	5.38 (0.78)	8.88 (0.62)	9.79 (0.74)	
	$R_t$	0.04 (0.01)	0.55 (0.21)	4.83 (0.64)	6.51 (0.52)	7.49 (0.39)	
	$R_{u/t}$	494%	145%	11%	36%	31%	26%
March11	$R_u$	2.50 (0.44)	2.13 (0.21)	2.79 (0.33)	5.27 (0.51)	10.24 (1.09)	
	$R_t$	0.77 (0.08)	0.94 (0.09)	1.07 (0.15)	2.40 (0.27)	4.17 (0.51)	
	$R_{u/t}$	223%	128%	162%	120%	145%	142%

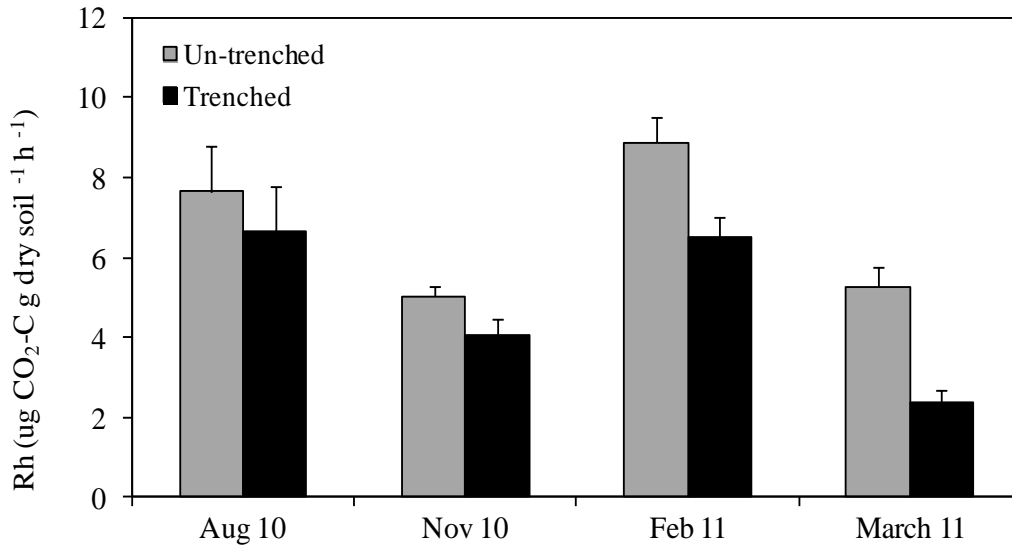


Figure 5.3.

Rates of heterotrophic respiration measured at 20°C with the respirometer for the trenched and un-trenched plots. Rates are averaged over the measurements of five soil samples for each of the trenched and un-trenched plots.

Respiration rates increased exponentially from lower to higher temperatures (Table 5.2). Rates measured using soil samples collected in March 2011 were slowest at most temperatures, closely followed by those measured for soils collected in November 2010. August 2010 and February 2011 soil collections presented similar rates of respiration for measurements conducted at or above 20°C.

Microbial biomass in soils of un-trenched plots was closely related to that of trenched plots (Figure 5.4). The slope of the linear relationship was  $<1$ . The relationship between microbial biomass in trenched and un-trenched plots was stronger in soils collected in the first four samplings (multiple  $R^2=0.9924$ ,  $P=0.003814$ ) than over the whole period (multiple  $R^2=0.9603$ ,  $P=0.003389$ ). The ratio of microbial biomass on trenched and un-trenched plots ( $MB_{u/t}$ ) was calculated as:

$$MB_{u/t} = \frac{(MB_u - MB_t)}{MB_t}$$

Where MB<sub>u</sub> is microbial biomass in un-trenched plots and MB<sub>t</sub> is microbial biomass on trenched plots. For each sampling, the values of MB<sub>u/t</sub> and R<sub>u/t</sub> were also strongly correlated (correlation coefficient of 0.9987,  $P=0.001297$ ).

The amounts of labile carbon were similar in both treatments at all times.

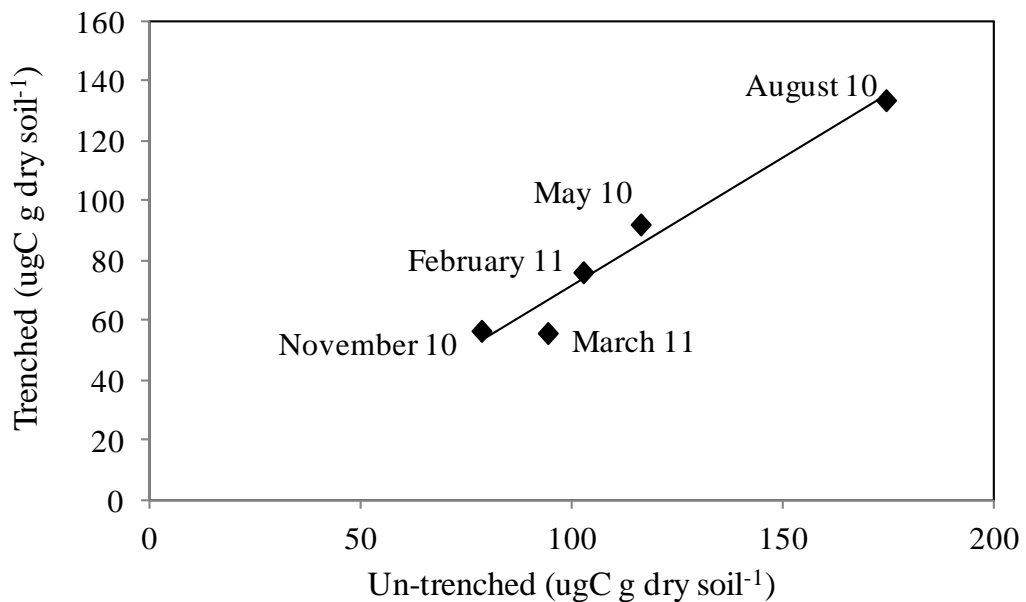


Figure 5.4.

Microbial biomass of trenched as a function of un-trenched plots. Each point is calculated from Chloroform fumigation and K<sub>2</sub>SO<sub>4</sub> extraction on three replicates of soil samples collected at each of the five trenched and un-trenched plots.

Q<sub>10</sub> for heterotrophic respiration were calculated from the exponential equation  $Q_{10} = e^{10b}$ . Q<sub>10</sub> was significantly greater in soils from the trenched plots than the un-trenched plots for all sampling dates (Table 5.3). Over time, Q<sub>10</sub> were similar across

the sampling months of August, November, and March for trenched and un-trenched plots. Soil samples for February 2011 had much higher  $Q_{10}$ , around twice those of previous samplings.

Table 5.3.

$Q_{10}$  values calculated from the respirometer measurements of heterotrophic respiration between 5°C and 25°C and respiratory quotient calculated at 20°C.

	Aug-10		Nov-10		Feb-11		Mar-11	
	$Q_{10}$	RQ	$Q_{10}$	RQ	$Q_{10}$	RQ	$Q_{10}$	RQ
Un-trenched	3.0 (0.6)	1.3 (0.2)	2.7 (0.9)	1.3 (0.3)	4.9 (1.2)	0.9 (0.3)	2.5 (0.6)	0.6 (0.2)
Trenched	3.5 (0.9)	1.9 (0.9)	3.7 (1.2)	1.6 (0.5)	7.4 (3.2)	1.2 (0.3)	3.5 (0.9)	0.6 (0.2)

Respiratory quotients for soils from trenched plots were always greater than those for un-trenched plots (Table 5.3). RQ values for both treatments were greater than 1 in August and November. There was then a decrease in RQ, suggesting a change in substrates. In February, RQ was close to 1, yet in March RQ was around 0.6 for both treatments.

#### ***5.4 Discussion***

Rhizosphere priming effects (RPE) were estimated to increase basal heterotrophic respiration by 54% over the course of the year-long study. Soil respiration in the field, heterotrophic respiration in the laboratory and microbial biomass were greater in un-trenched than trenched plots. Rhizosphere priming effects in un-trenched plots were greater than any priming effects induced by root decomposition in the trenched plots.

Priming effects can be generated by litter decomposition, including root decay. The ratio  $R_{u/t}$  was five to ten times lower in soils collected in August 2010 to February 2011 than in soils collected in March 2011. The difference was attributed to roots decomposition in trenched plots during the earlier months (Diaz-Pines et al. 2010). Yet despite increased respiration due to root decay, rates of heterotrophic respiration in un-trenched plots were faster than those in trenched plots. RPE was thus more powerful in stimulating SOM decomposition than priming effects induced by root decay. Clearly, in the present study, RPE is affecting SOM decomposition through increased microbial biomass. This contrasts with the results of Cheng (2009) who concluded that RPE stimulated the turnover of microbial populations rather than microbial biomass.

Soils collected in March yielded a larger estimate of RPE (ie. increasing basal respiration by 142%). Several factors suggested that effects of root decomposition had substantially diminished by March:

- In the field, respiration rates of trenched plots relative to un-trenched plots slowed in March compared to the previous months.
- The March 2011 sampling revealed a sharp and significant decrease in the microbial biomass of the trenched plots compared to their un-trenched counterparts, indicating a reduction in substrate availability - most probably roots of seedlings had decomposed.
- The respiratory quotient of soils from trenched plots decreased in March 2011 to the value of the un-trenched plots whilst it had been greater on the four previous occasions, confirming most fine roots had decayed.

Estimates of RPE from soils collected in March 2011 might thus be closer to true RPE and yet they might be conservative. Soil moisture in trenched plots was greater than in un-trenched plots in March 2011, contrary to previous months. In the field, soil respiration from trenched plots was linked to soil moisture. Laboratory measurements were made at field moisture. It is therefore likely that rates of respiration of soils from trenched plots were overestimated compared to rates that would be measured if soil moisture was the same as in un-trenched plots.

Rhizosphere priming effects decreased the temperature sensitivity of heterotrophic respiration as shown by the significantly greater  $Q_{10}$  of the soils from trenched plots. This result contrasts with those of Zhu and Cheng (2011). These authors concluded that RPE increased the sensitivity of SOM decomposition to changes in soil temperatures and that the level of RPE itself was dependant on soil temperatures. The effect of soil temperatures on the intensity of RPE could not be tested since the present experimental design relies on root free soils for measurements of soil respiration. A long-term field experiment using stable isotopes or growing trees in a control environment could elucidate the sensitivity of RPE to soil temperatures.

Autotrophic respiration was estimated to average just over 49% of total soil respiration of Mountain Ash, making heterotrophic respiration accounting for 51%. A positive relationship between respiration rates and soil temperatures for the trenched plots may contribute to a possible overestimation of the contribution of heterotrophs to soil respiration. Autotrophic respiration is usually thought to contribute from 24% to 81% of soil respiration (see Chapter 1). Experiments using

an interruption of supply of photosynthates to soil, such as trenching or girdling, refined these estimates to 36 to 65% (Bhupinderpal-Singh et al. 2003, Diaz-Pines et al. 2010, Frey et al. 2006, Hogberg et al. 2001, Jassal and Black 2006, Sayer and Tanner 2010). The estimate of autotrophic respiration derived here was calculated from measurements taken in the first three months of trenching and those of March 2011. Sayer and Tanner (2010) showed that heterotrophic respiration can be estimated by measuring respiration immediately before and up to five days after trenching and that results are similar to that measured one year later, after roots on the trenched plots have decomposed. Fine root decomposition can last from several months to more than two years after trenching and thus interfere with the process of isolating heterotrophic and autotrophic respiration (Epron et al. 1999, Sayer and Tanner 2010). It appears that roots of Mountain Ash seedlings did not start decomposing for the first few months post-trenching and their root system had decomposed more or less entirely by March 2011.

### ***5.5 Conclusion***

This study showed that trenching experiments have one very important limitation, the lack of consideration to the rhizosphere priming effect, when used to estimate the contribution of autotrophic and heterotrophic respiration to soil respiration. Priming effect was estimated to reach close to 140% and was so intense that it could be detected despite the clear impact of root decomposition in the trenched plots. RPE translated into an increase in microbial biomass and increased temperature sensitivity of heterotrophic soil respiration.



The current results on rhizosphere priming effect should be validated by using a method using isotopic composition of the CO<sub>2</sub> respired by both trenched and un-trenched plots.

## *Chapter 6*

### *Increase of litter input to soil and belowground respiration*

#### *6.1 Introduction*

Net primary productivity (NPP) is likely to be enhanced under predicted climate scenarios of increased atmospheric [CO<sub>2</sub>] and temperature. Increased atmospheric [CO<sub>2</sub>] can increase rates of photosynthesis and hence plant growth (Norby et al. 1999). Warmer temperatures may contribute to lengthening of the growing seasons of grasses (Niu and Wan 2008, Wan et al. 2005) and woody species such as those of northern Europe (Morales 2007). A meta analysis of effects of elevated [CO<sub>2</sub>] predicted plant growth to increase by a little over 50% for grassy species and over 40% for woody species (Zak et al. 2000). The associated increase in litter production may however contribute to an increase in heterotrophic respiration due to larger amounts of organic matter being available to heterotrophs (Deng et al. 2010, Zak et al. 2000). Moreover, an increase in temperature is also likely to enhance litter production and decomposition (Schindlbacher et al. 2009).

It has been suggested that tissues of plants growing in atmospheres enriched in CO<sub>2</sub> have reduced nitrogen (N) concentrations but are richer in lignin than those of plants growing at ambient [CO<sub>2</sub>] (Henry et al. 2005, Knops et al. 2007). This too could affect decomposition and heterotrophic respiration due to their negative relationship with C:N ratios, lignin content and lignin:N ratios, and positive relationships with N concentrations (Melillo et al. 1989, Melillo et al. 1982). In the long term, changes in litter chemical properties may alter nutrient and C contents of soils. Consequently, soil respiration is often N-limited (Jones 1998) and will also be subject to indirect

change by atmospheric [CO<sub>2</sub>]. Studies on litter decomposition using litter bags in elevated [CO<sub>2</sub>] atmospheres have, however, not always recorded a change in specific decomposition rates, even after two years (Henry et al. 2005, Knops et al. 2007).

Addition to soil of substrates such as litter or glucose leads to an increase in respiration which is greater than that suggested by the amount of substrate alone (Crow et al. 2009, Hamer and Marschner 2005, Nottingham et al. 2009, Prevost-Boure et al. 2010, Sulzman et al. 2005). This extra release of CO<sub>2</sub>-C is attributed to the ‘priming effect’, named by Bingeman et al. (1953), and recently defined as “strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil” (Kuzyakov et al. 2000). Priming effects can also be negative resulting in slower rates of respiration (Blagodatskaya and Kuzyakov 2008, Kuzyakov et al. 2000). The direction of priming effects (i.e. positive or negative) is directly dependent on:

- the C:N ratio of the active SOM pool (Kuzyakov et al. 2000)
- the nutrient composition of soils (Kuzyakov et al. 2000)
- the amount of organic carbon added relative to the carbon contained in soil microorganisms (Blagodatskaya and Kuzyakov 2008).

Due to limitations in technology and the difficulty of separating components of soil respiration (autotrophic and heterotrophic), most studies have focussed on detecting and quantifying priming effects rather than identifying governing mechanisms.

Several mechanisms have been proposed as driving priming effect. The most widely accepted is that of co-metabolism. Co-metabolism is the process by which the addition of substrate provides the energy necessary for microbes to produce the

enzymes required for decomposition of organic matter, including the added substrate and recalcitrant or stable SOM (Kuzyakov et al. 2000). Fontaine et al (2003) complemented the co-metabolism theory by suggesting that addition of substrate to soil first stimulates the activity of specialized and fast-growing microorganisms, commonly referred to as r-strategists, that are otherwise dormant since they cannot metabolize SOM. Once r-strategists have exhausted the higher energetic compounds rich in labile C found in the added substrate, they either die or enter a dormant stage. Slow-growing K-strategists then establish and use more recalcitrant components of the substrate. They are only stimulated toward the end of substrate decomposition; their role is only important if the substrate contains polymerised compounds that r-strategists cannot use. Since microorganisms release enzymes that contribute to the degradation of organic matter and K-strategists are more responsive to refractory compounds, the enzymes they synthesize to degrade added substrates may also have an impact on SOM decomposition. Therefore, more chemically rich substrates added to the soil tend to broaden the spectrum of synthesized enzymes and, hence, the likelihood of both impact on litter decomposition and respiration of SOM (De Nobili et al. 2001, Fontaine et al. 2003).

The composition of substrates added to soil determines the extent to which K-strategist microorganisms can participate in the increase of CO<sub>2</sub>-C efflux from amended soils and how long a response will be observed. Litter, containing polymerised substances, has a longer residence time in soil than purified simple substance such as glucose, and thus facilitates the growth of the K-strategist population and their release of SOM decomposing enzymes. This hypothesis is based on a range of studies including a number of experiments using simple sugars such as sucrose and fructose as substrates. Other studies have shown that priming effects can

be caused by addition of sugar C (Hamer and Marschner 2005, Nottingham et al. 2009, Wu et al. 1993).

Respiratory quotients (RQ) are defined as the number of moles of CO<sub>2</sub> produced for every mole of O<sub>2</sub> consumed ( $RQ = RCO_2 / RO_2$ ). The value of RQ depends on the origin of the C substrates available for respiration (Dilly 2001). When simple carbohydrates are decomposed, RQ is 1. A low RQ (<1) is typically associated with the metabolism of more refractory compounds. A high RQ (>1) indicates metabolism of readily available organic acids (e.g. citric and oxalic acids) or other compounds that are more oxidised than sucrose (Dilly 2003). Novel technology measuring both CO<sub>2</sub> effluxes from and O<sub>2</sub> intakes by soils facilitate the calculation of RQ and may contribute to the determination of the origin of soil respired CO<sub>2</sub>.

Here I examine the influence of litter on soil C cycling using field- and laboratory-based studies. Soils from the Australian High Country were manipulated through long-term addition and removal of litter. I hypothesised that soil respiration would be positively correlated with amounts of litter in the field and that the effect would be increased with time as litter decomposed. The laboratory experiment examined rates of respiration under controlled conditions and standardised amounts of litter addition. I used a novel system able to measure both heterotrophic soil respiration and O<sub>2</sub> uptake allowing calculation of the RQ of the incubated soils.

## ***6.2 Materials and methods***

The experimental site is located in the Snowy Plains (36°06'05"S 148°31'35"E) of the Snowy Mountains in New South Wales, Australia at an altitude comprised between

1475 m and 1700 m above sea level (see Chapter 4). The mean annual minimum temperature at the study site is 1.7 °C and the maximum 10.2 °C. Average annual precipitation is ~ 1600 mm. Sites are snow covered for approximately three months of the year from July to September.

Three sod tussock grassland sites (20 m x 20 m) and three sub-alpine woodland sites (40 m x 40 m) were established on private property used in the summer time for cattle grazing. The sites chosen for this study are fenced to exclude cattle. The grassland sites are dominated by grasses *Poa* Spp and the woodland sites are dominated by *Eucalyptus pauciflora* (snow gum) with an understorey dominated by woody shrubs such as *Bossiaea foliosa* (N-fixing), *Leucopogon* spp. (Ericaceous) and *Tasmannia xerophila* with patches of grasses such as *Poa* spp.

Soils are derived from Silurian Granodiorite, approximately 433 ±1.5 million years and are alpine humus (Costin, 1962) with a upper layer rich in organic matter. The pH averaged 5.0 for the woodland soils and 5.3 for the grassland soils. Total carbon is 8.4 % for a C:N ratio of 7.69 in the woodland soil and 4.1 % for a C:N ratio of 13.82 in the grassland soil.

### 6.2.1 Field experiment

At each of the three woodland sites, five collars (50 mm tall x 200 mm diameter) were installed during October 2009 and measured baseline soil respiration for five months. During late February 2010, three sets of two quadrats (1 m<sup>2</sup>) were marked in each of the woodland sites (n=3), nearby previously installed collars. Litter was raked from one quadrat and applied to the adjacent quadrat, thus creating a 'no litter

treatment', a 'double litter' treatment and 'control' using the previously installed collar. A collar (50 mm tall, 200 mm diameter) was inserted into the soil at the centre of each quadrat, and maintained in position by alloy pegs.

Litter was also sampled in an adjacent area (1 m<sup>2</sup>) and was dried at 60 °C and weighed for biomass estimates.

Soil respiration was measured at monthly interval starting one month after the litter manipulation. Measurements were taken during the active growing season from March 2010 to May 2010 then resumed from October 2010 to March 2011.

Soil respiration was measured using Vaisala carbocap GMP343 (Vaisala, Helsinki, Finland) mounted on a 5.25 l lid and linked to a Vaisala MI70 data logger. At each site, the Vaisala was calibrated for atmospheric pressure and relative humidity measured with a Kestrel 3500 mini weather station. The lid is placed on the collar and the lid-Vaisala-collar association works as a static chamber. The lid was left on the collars for 10 minutes out of which only the last eight were considered for calculation of the rate of CO<sub>2</sub> efflux. The recording during the first 2 minutes was discarded in order to allow the [CO<sub>2</sub>] to stabilise. Respiration rates were calculated from the linear increase in [CO<sub>2</sub>] recorded by the Vaisala. Soil temperature at 100 mm depth and soil volumetric water content were recorded adjacent to collars at the time of measurement.

During March 2011, soil was sampled from 0-100 mm in each of the woodland and grassland plots. One core was taken from each 'double litter' and 'no litter' plot. Samples were sieved and bulked according to the treatment and plot they were

collected from (i.e. woodland controls, woodland “+” litter, woodland “-” litter and grassland, n=3 per treatment). Microbial biomass C and labile C were determined from these samples using the fumigation-extraction method (Ohlinger 1995). Subsamples were fumigated for 48 hours and C content determined by Mn(III) assay, at an absorbance of 495 nm. Microbial biomass was calculated assuming a  $k_{ec}$  of 0.35 and labile carbon was determined from non-fumigated samples.

### *6.2.2 Laboratory experiment*

In March 2011, three 100 mm deep cores of soil were taken from each of the woodland (n =3) and each of the grassland (n =3). The soils were sieved to 2 mm and bulked by soil type. Gravimetric water content was 46% for the woodland soil and 38% for the grassland soil. Three sets of soils were isolated for each woodland and grassland soils. The first set was kept as a control. They are referred to as W for the woodland controls and G for the grassland controls. I added 0.7 g woodland litter/100 g woodland soil dry weight (WL) and 0.16 g grassland litter/100 g grassland soil dry weight (GL) to a second set and doubled the amount of litter for two more sets 1.4 g/100 g soil for woodland soils (W2L) and 0.32 g litter/100 g grassland soil (G2L). The lower amounts of litter correspond to those measured in the field on a comprehensive survey (Jenkins, personal communication). The litter (<5 mm) was collected adjacent to the plots from three random quadrats of an area of 1 m<sup>2</sup> for the grasslands and 2 m<sup>2</sup> in the case of the woodlands. The two types of litter were kept separated, dried at 65°C for three days and ground to a powder (<0.75 mm). Total nitrogen and carbon in ground litter were measured using combustion analysis (Elementar Vario Max CNS, Analysensysteme GmbH). All other nutrients were determined by nitric acid digestion followed by measurement by ICP-AES



(Table 6.1). Respiration was measured at 20°C within an hour of adding the ground litter and then 1, 3, 7, 10, 16, 25, 50 and 110 days after litter addition. The soils were incubated at 20°C between measurements. After 16 days of incubation, respiration was measured at different temperatures, ie 5-10-15-20-25°C, corresponding to soil temperatures recorded in the field from early Spring to late Autumn.

Soil respiration was measured using a ‘respirometer’ as described in Jenkins and Adams (2010): 100 g of fresh soil sieved to 2 mm was added to polycarbonate cylinder connected to an infra-red gas analyser (IRGA) and differential oxygen analyser (DOX) for CO<sub>2</sub> and O<sub>2</sub> analysis. The tubes were maintained at constant temperatures in a water bath during and up to an hour before measurements. Respiration rates were calculated over 60 seconds after CO<sub>2</sub> concentration had remained steady for three minutes.

### *6.2.3 Statistical analysis*

All data analysis were performed in R. Repeated measure ANOVA, with time and litter treatments as factors, were applied to data collected from the field. One-way ANOVA was used for each measurement to compare soil respiration, soil temperature and soil moisture. Two way Student t-tests, with litter amount as a factor, was used for each laboratory measurement and corresponding RQ.

Table 6.1.

Amount of elements added to 100 g dry weight of grassland and woodland soils when 0.16 g of grassland litter (GL) and 0.7g of woodland litter (WL) were added to either soil.

Nutrients	Grassland Litter	Woodland Litter
Nitrogen (N)	2.1	6.2
Carbon (C)	57.0	335.1
C:N	27.1	54.0
Phosphorus	0.1	0.6
Potassium           mg/100g DW soil	0.4	1.2
Sulphur	0.2	0.7
Sodium	0.0	0.1
Calcium	0.6	8.4
Magnesium	0.3	1.0
Copper	2.2	36.2
Zinc	1.1	19.9
Manganese           ppm	6.7	38.3
Iron	96.0	134.0
Boron	0.1	0.7

### 6.3 Results

An average of 4.8 t ha<sup>-1</sup> or 480 ± 154 g m<sup>-2</sup> was added to the double litter plots and therefore removed from the no litter plots.

#### 6.3.1 Litter effect

The litter manipulation had no effect on soil respiration in the field at any time (Figure 6.1, RM ANOVA,  $P > 0.05$ ). The respiration rates of both no litter and double litter plots followed a similar pattern to rates of the control plots. Neither soil

volumetric water content nor soil temperatures differed significantly between treatments (Figure 6.2, RM ANOVA,  $P>0.05$ ). There was little difference in microbial biomass or labile carbon of the soils sampled in March 2011 (Figure 6.3) and it was not significant for any of the treatments (ANOVA,  $P>0.05$ ).

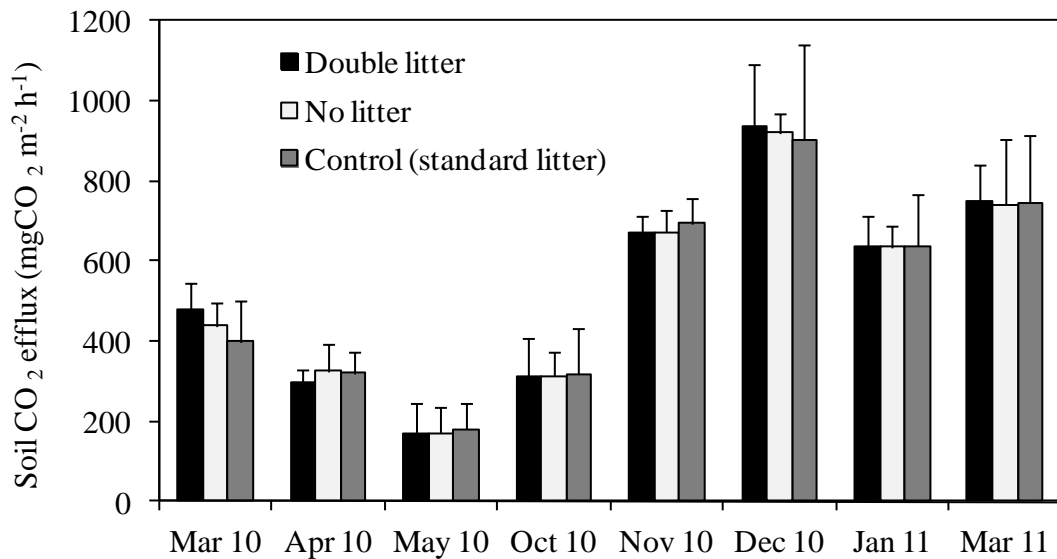


Figure 6.1.

Soil respiration from the controls, no litter and double litter plots measured on site at the Snowy Plain.

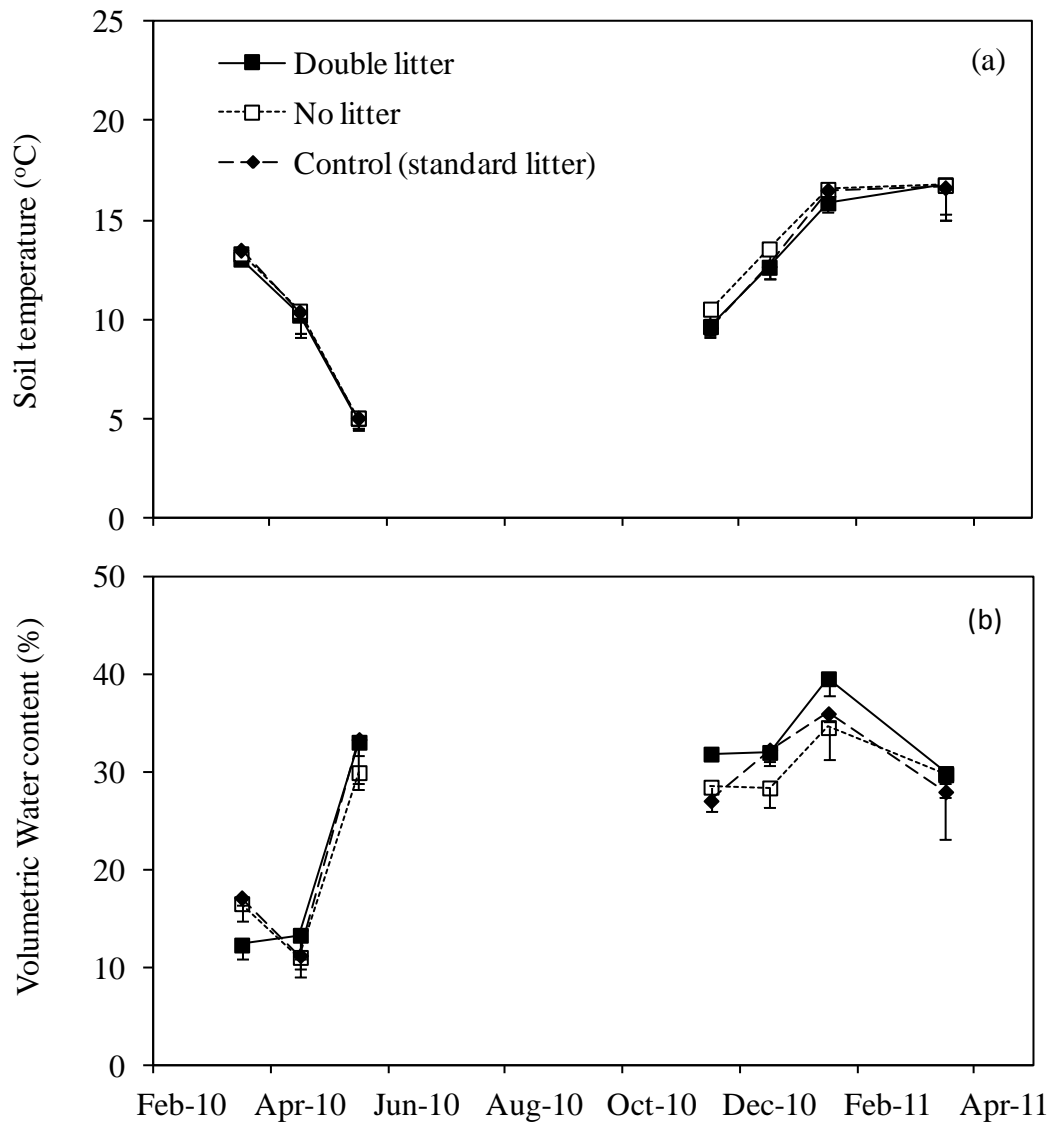


Figure 6.2.

Soil temperature (a) and soil volumetric water content (b) measured in the field on the control (standard litter), double litter and no litter plots.

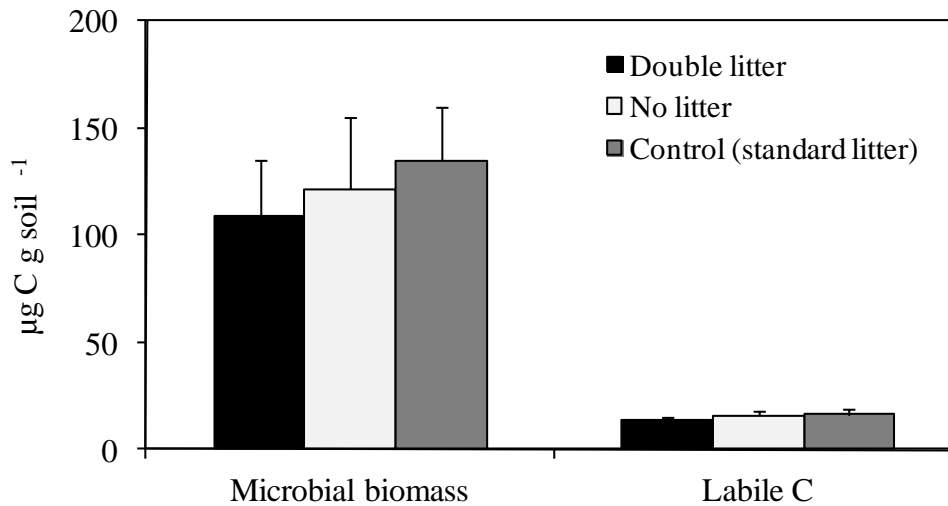


Figure 6.3.

Microbial biomass and labile carbon measured after Chloroform fumigation and  $\text{K}_2\text{SO}_4$  extraction from soil samples collected in March 2011, one year after manipulation.

In the laboratory, the addition of litter to both woodland and grassland soils had an immediate positive effect on the rates of respiration (Figure 6.4). One hour after adding litter, rates of respiration in woodland soils had increased by 100% in the WL treatment and a further 150% in the W2L treatment. Rates of respiration were significantly different between all treatments one hour after litter addition to the woodland soils (t-test,  $P < 0.05$ ).

The litter-promoted increase in respiration lasted throughout the 110 days of incubation at both rates of addition although the amplitude of the response diminished over time. Of the nine measurements, the difference between WL treatment and control was significantly greater for six of the first seven measurements up until Day 25. The only exception was on Day 10 (t-test,  $P < 0.05$ ). In the case of the W2L treatments, the increase in respiration compared to the control

soils was significant for all but two of the measurements, 24 hours and 110 days after litter addition (t-test,  $P < 0.05$ ).

The amplitude of the immediate response of the grassland soils to the litter addition was greater than that of the woodland soils. One hour after addition, rates of respiration of the GL soils were more than 100% greater than that of the control soils (G soils) whilst the increase for the G2L soils was almost 200% greater. The response of the grassland soils decreased within 24 hours and was only significant on one occasion, 16 days after litter addition, for the GL soils and 3, 10, 16 and 50 days after addition in the case of the G2L soils.

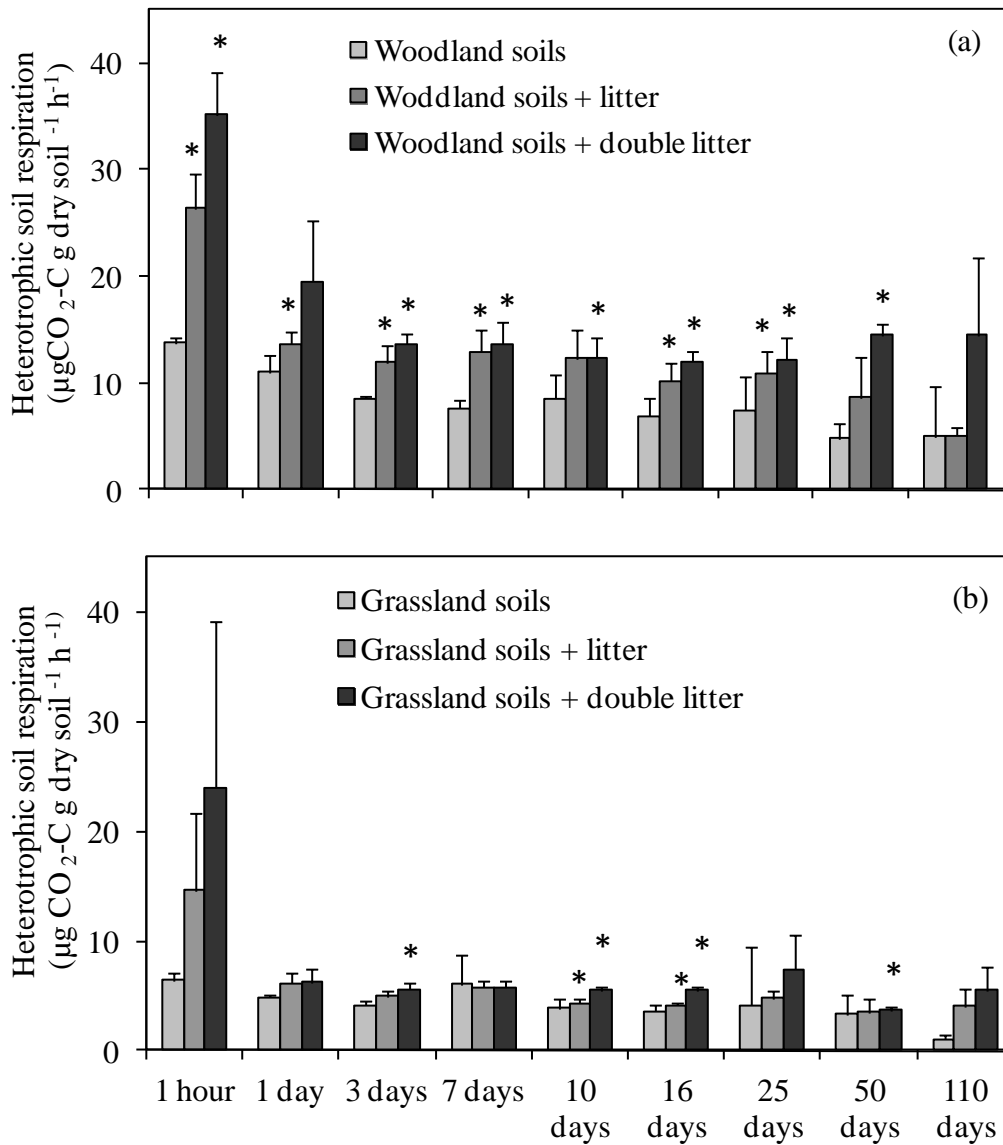


Figure 6.4.

Heterotrophic soil respiration measured on the respirometer in the laboratory for woodland (a) and grassland (b) soils. Litter amendments are 0.7 g woodland litter/100g DW woodland soil and 0.16 g grassland litter/100g DW grassland soil. Double litter amendments are addition of 1.4 g woodland litter/100g DW woodland soil and 0.32 g grassland litter/100g DW grassland soil. “\*” indicates significant difference to the non amended soils ( $P < 0.05$ )

After 16 days of incubation, measurements were conducted at a series of soil temperature ranging from 5 to 25°C (Figure 6.5). Respiration rates increased with temperature regardless of the treatment. The calculated  $R^2$  of the relation between respiration rate and temperature was over 0.98 for all treatment except the control

grassland for which  $R^2$  was 0.86 and the  $P$ -values of the linear regression between the two variables were all  $<0.05$  (Table 6.2).

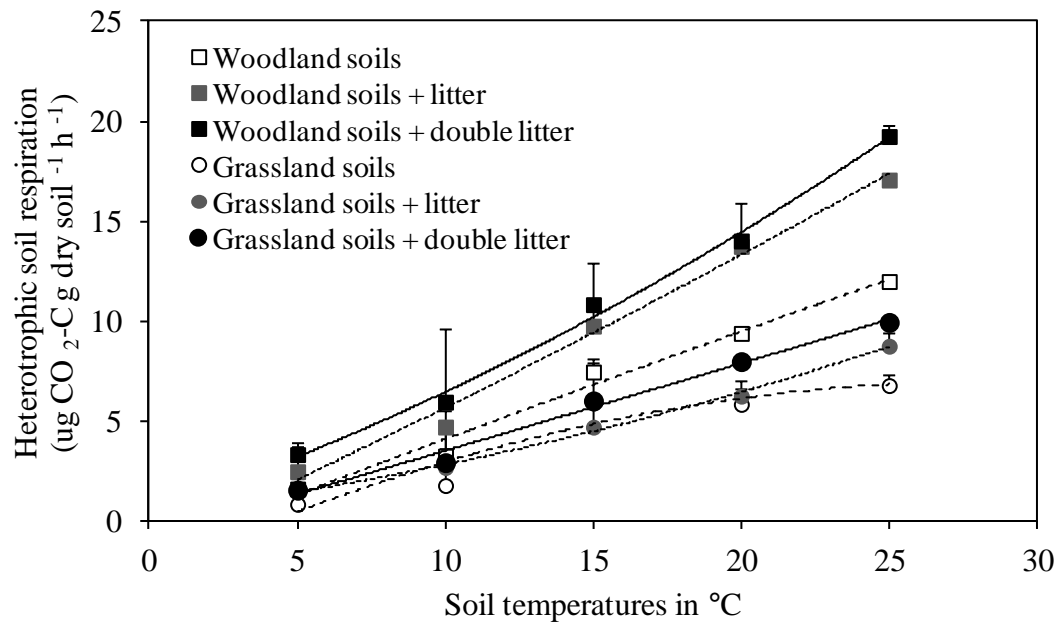


Figure 6.5.

Heterotrophic soil respiration measured in the laboratory at 5, 10, 15, 20 and 25°C. on day 16 of litter incubation with fitted curves. Litter amendments are 0.7 g woodland litter/100g DW woodland soil and 0.16 g grassland litter/100g DW grassland soil. Double litter amendments are addition of 1.4 g woodland litter/100g DW woodland soil and 0.32 g grassland litter/100g DW grassland soil.



Table 6.2.

R square and *P*-value for linear regression of rate of heterotrophic soil respiration measured in the laboratory at 5, 10, 15, 20 and 25°C on day 16 of litter incubation and for grassland and woodland soils with various amount of litter addition.

Treatment	R <sup>2</sup>	p-value
Woodland soils	0.9833	0.00092
Woodland soils + litter	0.9893	0.00047
Woodland soils + double litter	0.9892	0.00047
Grassland soils	0.8637	0.02230
Grassland soils + litter	0.9875	0.00059
Grassland soils + double litter	0.9892	0.00048

### 6.3.2 Cumulative respiration and priming effect

Analysis of cumulative amount of CO<sub>2</sub> released suggests clearly that, without added litter, rates of respiration slow over time as substrates become more limiting (Figure 6.6).

The priming effect was calculated as follows:

(Cumulative respiration of soil + litter) – (Cumulative basal respiration of the controls + C added by litter).

All respiration values were those calculated over the length of the incubation period (i.e. 110 days). Priming effect was responsible for the release of 4 mg of CO<sub>2</sub>-C g dry soil<sup>-1</sup> from the WL soil, 15 mg CO<sub>2</sub>-C g dry soil<sup>-1</sup> from the W2L soils corresponding respectively to 26% and 95% of the basal respiration measured from the W soils. Priming effects in grassland soils released 3 mg CO<sub>2</sub>-C g dry soil<sup>-1</sup> from the GL soils and 5 mg CO<sub>2</sub>-C g dry soil<sup>-1</sup> from the G2L soils, or roughly 140% and 170% of the basal respiration measured for G soils.

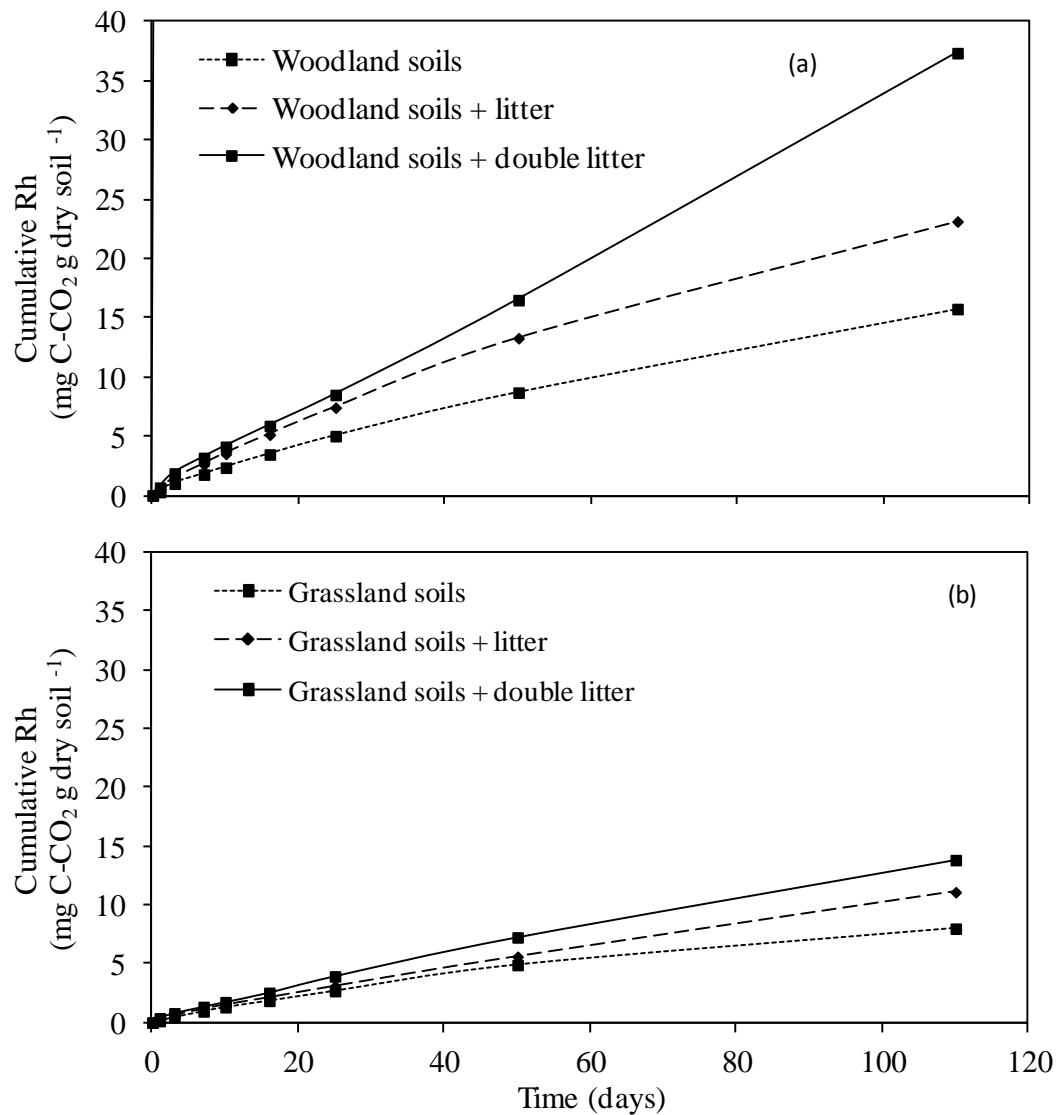


Figure 6.6.

Cumulative heterotrophic soil respiration from woodland (a) and grassland soils (b). Litter amendments are 0.7 g woodland litter/100g DW woodland soil and 0.16 g grassland litter/100g DW grassland soil. Double litter amendments are addition of 1.4 g woodland litter/100g DW woodland soil and 0.32 g grassland litter/100g DW grassland soil.

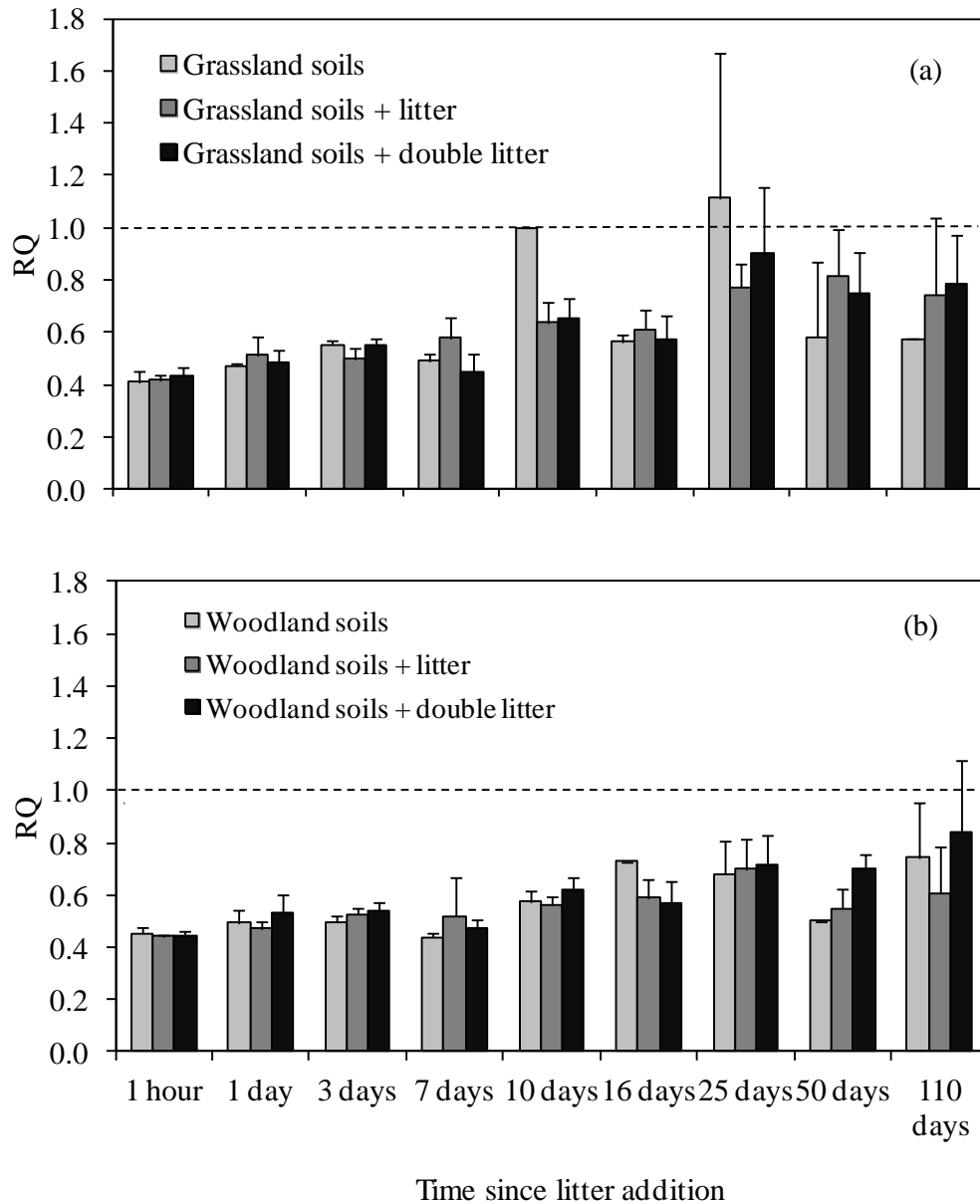


Figure 6.7.

Respiratory quotient calculated as the number of moles of CO<sub>2</sub> released divided by the number of moles of O<sub>2</sub> absorbed during respiration measurements of the grassland soils (a) and woodland soils (b) with no litter, single addition and double addition of litter.

### 6.3.3 Respiratory quotients

Respiratory quotients (RQ) for all treatments and both soil types were between 0.4 and 0.6 until Day 16. Except for two outliers on Day 10 and 25, RQ of grassland

controls were ~0.6 for the 110 days of incubation. From Day 25, RQ from grassland treatments increased to about 0.8. For both soil types the amount of litter added did not affect RQ except in the woodland soils at Day 50 and Day 110 when W2L soils had a greater RQ than WL soils.

## ***6.4 Discussion***

Litter amendments of subalpine soils from the Snowy Mountains strongly increased heterotrophic respiration in the laboratory manipulations but not in field trials.

### *6.4.1 Litter addition and heterotrophic respiration*

In the laboratory, litter addition had an immediate positive impact on heterotrophic respiration (one hour after soils were amended with litter) such that the fastest respiration rates were measured at this time (over the 110 days). Lags of one day (Hamer and Marschner 2005) and up to six days (Nottingham et al. 2009) between substrate addition and response of soil respiration have been previously reported. The immediate response to the addition of litter of respiration rates in grassland soils were proportionally greater than those of woodland soils, despite the lesser amounts of grassland litter. The grassland litter contained only 17% of the C and 30% of the N contained in woodland litter. The response to litter input was, however, more sustained in the case of the woodland soils. This could be due to a fraction of woodland litter carbon being in forms such as aromatic components that are very refractory (Dilly 2001) and thus released at a slow rate. This is borne out by C:N ratios that suggest woodland litter (C:N=54) would overall be more refractory than the grassland litter (C:N=27.1). C:N ratios of grassland litter suggests that some

carbon was in a form that is readily available and quickly used by r-strategist microorganisms. Impacts of litter addition were thus more intense but shorter lived in the grassland soils.

In contrast, the more refractory woodland litter provides opportunity for K-strategist populations to grow and be sustained for the entire incubation period.

#### *6.4.2 Priming effect*

The soils amended with litter presented an increase in respiration originating both from litter decomposition and a priming effect. The priming effect accounted for an extra 26% release of CO<sub>2</sub> for the WL treatment (above basal rates of respiration) and 95% for the W2L treatment. It similarly accounted for an extra 139% for the GL treatment and 173% for the G2L treatment. These values are likely an underestimation of priming, as not all the C added via additions of litter were mineralized by the end of the incubation period. For woodland soils, the intensity of the priming effect was positively correlated with the amount of litter added. The doubling of woodland litter more than trebled the amount of CO<sub>2</sub>-C released by the woodland soils originating from the priming effect. On the other hand, the doubling of grassland litter resulted in less than a doubling of extra CO<sub>2</sub>-C production in the grassland soils. Recent studies have shown that the complexity of the substrate has a marked effect on the intensity of priming. The combined addition of two substrates resulted in a greater priming effect than the additive effect of addition of the same substrates separately (Hamer and Marschner 2005) whereas soil amendment with root extracts yielded a greater priming effect than amendment with glucose or amino acids (De Nobili et al. 2001).

Both the direction (negative or positive) of the priming effect and its intensity are dependent on the nature of the substrates, and the nutrient composition of soils, and the C:N ratio of the SOM pool (Kuzyakov et al. 2000). Here, although grassland litter (with a lower C:N) produced a greater priming effect than the woodland litter at field stocking rate, the doubling of the input did not produce a proportional response. This may be a result of the greater C:N ratio in grassland soils. Indeed the C:N ratio of the grassland soils at ~14 is roughly twice that of the woodland soils at 7.7. Based on treatment effects observed in this study, any increase in NPP and subsequent litter production (e.g. as a consequence of changes in climate) will not translate into a similar increase in priming of respiration in grassland and woodland soils of the Snowy Plains.

RQ were low ( $\ll 1$ ) during the whole incubation period. This indicates a heavy use of  $O_2$  during the process of respiration of recalcitrant C. Respiration acts upon several pools of carbon, each requiring different amounts of oxidation and energy. The r and K-strategist theory (Fontaine et al. 2003) suggests initial use of easily degradable forms of C at  $RQ \geq 1$ . RQ in the present experiment suggest more recalcitrant forms of C were being used as substrates soon after litter addition. Nottingham et al (2009) detected a priming effect after sucrose and maize addition. Their peak of 'priming' release of  $CO_2$  was followed by a peak release of  $CO_2$  derived from substrate decomposition, then a sustained  $CO_2$  efflux originating from priming. The consistently low RQ values observed in this study suggests that sudden increases in respiration are consequences of both litter decomposition and SOM decomposition.

The capacity of soils of the High Country of Australia to be C sinks and the strength of the sink may be reduced by a continuous, climate-fuelled increase in primary production that will ensure constant and increased litter inputs to soil. Repeated substrate addition to soil leads to a greater increase in CO<sub>2</sub> efflux than a similar amount of substrate added in a single dose (De Nobili et al. 2001, Hamer and Marschner 2005). Repeated substrate addition can maintain priming effects in C rich soils, such as those of cool and alpine areas, for many years (Sayer et al. 2011). Even irregular litter inputs, once triggered, can sustain priming effects for months after the original substrate has fully decomposed (Fontaine et al 2011).

In coming decades, soil CO<sub>2</sub> efflux from the Australian high country should increase due both to the enhanced input from litter decomposition and associated priming effects. The chemical nature of the C inputs and the availability of other key minerals, such as N and P, determine whether recalcitrant pools of soil C grow or become depleted (Fontaine et al. 2011). C originating from litter decomposition has a relatively short turnover time (Schlesinger and Lichter 2001) and priming effects can be fuelled by recalcitrant forms of carbon (Heimann and Reichstein 2008, Sayer et al. 2011). In the long term, this may lead to a depletion of soil carbon.

#### *6.4.3 Field study*

Results of laboratory studies do not always correlate with results of field studies as was the case in the present study. Indeed Prevost-Bouré et al (2010) observed an effect of litter treatment in the field but not in the laboratory. Similarly, the removal of root activity from field plots artificially enriched in litter, reduced or even cancelled the priming effect (Subke et al. 2004). There is growing evidence that

positive feedbacks between rhizosphere activity and litter decomposition governs the presence and intensity of priming effects created by litter input (Prevost-Boure et al. 2010, Schaefer et al. 2009, Subke et al. 2004).

The lack of response of soil respiration to litter treatment for the sites studied here may be the result of several factors. First, rates of decomposition are very slow in cold climates such as the Snowy Plains. During the winter months, study sites are snow-covered and monthly measures in spring, summer and autumn months show that soil temperatures range from 5°C to 16.8°C. Secondly, the dominant plant species in the woodland, *Eucalyptus pauciflora* (snow gum) and *Bossia foliosa* are sclerophyllous. The tough epidermis of leaves slows their decomposition. Litter added to the woodland plots in the Snowy Plain contain woody materials that have much slower rates of decomposition than grassland leaf litter, for example. Overall, litter additions in the field, where whole leaves, pieces of bark, twigs and other highly refractory material are added, presents very different responses to laboratory additions of ground leaf litter.

#### *6.4.4 Temperature effect*

Measurements on Day 16 of incubation at a range of temperatures from 5 °C to 25 °C, confirmed that respiration rates increase with temperature regardless of litter addition. At any given temperature, respiration rates were greater in amended soils than in the control, albeit not significantly so. Predictions of increased temperatures in future have a general corollary of increased rates of decomposition of litter and litter and soil respiration (Schindlbacher et al. 2009) especially in colder climate such as those at high altitude.



The high country of Australia includes large areas of inverted tree lines, where air temperatures in the bottom of valleys are much less than on the slopes. Often, temperatures on the flat bottom of valleys fall well below freezing, preventing the growth of trees and favouring grasses that offer protected meristematic tissues. At the local scale, global warming might translate into an increase of the temperatures in the valleys that could, in turn, lead to a colonisation of grasslands by trees. The resulting change in vegetation cover would most probably alter the soil carbon dynamics of the local ecosystems (Jackson et al. 2002, Jenkins and Adams 2010). Moreover, the transition period from grassland to woodland may change both soil characteristics and above ground carbon and nutrients inputs. As demonstrated above, soil stored carbon may be compromised.

### ***6.5 Conclusion***

In light of the present results and the current state of the research, it is predicted that the heterotrophic component of soil respiration will increase in the coming decades in the Australian High Country. However, the extent of this increase will depend on the degree of changes in climate that will be experienced at the regional scale and, as a consequence, the increase in primary production and the quantity of litter generated. More research is needed on the relations among soil temperature, litter decomposition and soil respiration. Longer-term field-based experiments are required to quantify accurately the extent and consequences of priming effect and the level of interaction between free living microorganisms and the rhizosphere. The use of isotopic methods could improve the understanding of the mechanisms in action.

## ***Chapter 7***

### ***Conclusion***

The previous chapters have shown that climate change cannot be reduced to elevated atmospheric [CO<sub>2</sub>], elevated temperatures or a change in rainfall patterns when studying its impacts on soil respiration. Climate also affects soil respiration indirectly through changes in natural conditions that may trigger a change in primary productivity or foster major disturbances such as wildfires. This chapter provides a synthesis of previous chapters, presenting the main findings of this thesis, as well as providing an overview of technical issues in measuring and partitioning soil respiration. The results presented in this thesis are also placed in a global context.

#### ***7.1 Techniques for measuring soil respiration***

Various techniques were used to measure soil respiration and separate its autotrophic and heterotrophic components.

Static chambers were chosen for field measurements. Multiple measurements of several small areas within a plot or research site, using collars or chambers, are still very widely used to determine rates of soil respiration within a wide range of ecosystems. A key limitation of this approach is the risk of over or under-estimation of respiration rates because of gradients in respiration in relation to location of dominant overstorey trees whereby respiration rates are faster closer to trees and diminish with increasing distance from the trees. Between about 1 and 1.5 m from tree stems, respiration rates slow exponentially (Baldocchi et al. 2006, Saiz et al. 2006). At the HFE, for example, switching from portable static chambers to using the

whole underfloor of the whole tree-chambers (WTC) led to recording respiration rates that were 38.5% faster on average. The uncertainty of estimation of rates of soil respiration for whole ecosystems based on point measurements has a clear dependence on tree stocking rates as well as intensity of sampling.

Many of the designs used to partition soil respiration into its main components and quantify the contribution of each component are ecosystem-specific. For example, girdling has proved to be successful when used on Scots pines (*Pinus sylvestris*, Bhupinderpal-Singh et al. 2003, Hogberg et al. 2001). However, when used in Eucalyptus stands, the release of reserves of carbohydrates contained in roots delayed the effect of the girdling on soil respiration by six months (Binkley et al. 2006). The decrease in soil respiration after this period was still lower than observed when girdling Scots pines and there was no decrease in fine root biomass or fine root respiration. Girdling approaches require a more or less immediate reduction in root and mycorrhizal respiration to be successful (i.e. to be able to quantify the separate contributions of different processes), and girdling is clearly not a useful approach for eucalypts or resprouting trees (Binkley et al. 2006).

The mesh-collar chambers that were used successfully by Heinemeyer et al (2007) are another example of the specificity of experimental designs to particular ecosystems. Similar mesh-collar chambers did not deliver the expected partitioning of the components of belowground respiration at HFE (Chapter 2). Both experimental sites were plantations and both had no understorey. Pine trees are known for their shallow roots whereas, although many roots of the *Eucalyptus saligna* at HFE grew at less than 30 cm depth, the collars did not reach deeper than

25 cm and more roots grew at depth estimated to be up to 4.5 metres (Duursma et al. 2011). The collars were thus rendered inefficient for the purpose of eliminating root contributions to soil respiration. Moreover, inspection of the soil within the collars showed that roots survived for up to nine months after being severed from trees via the installation process. Root decay was very slow and roots remained alive for several months after being severed. The study at the HFE confirmed the results of Binkley et al (2006) and indicated that the use of mesh-collar chambers needs special consideration when applied to eucalypts. For example, mesh-collar chambers might be used in plantations with no understorey, in well-watered areas, provided they are installed at the time seedlings are planted. The depth of rooting should be monitored in order to determine when roots start growing deeper than the wall of the collars. From this time onward, respiration rates measured from within the mesh collars should be considered to be partly fuelled by root respiration.

Chapter 5 of this thesis suggests that previous estimates of the ratio of autotrophic: heterotrophic respiration calculated using the ‘trenching method’ might have under-estimated heterotrophic components due to a rhizosphere priming effects (RPE). Heterotrophic respiration was estimated to be 51% of total respiration. However, RPE increased basal heterotrophic respiration as much as 142% in un-trenched plots supporting seedlings. Clearly when estimated via trenching, the autotrophic component of soil respiration comprised root and rhizosphere respiration as well as that generated by RPE. Estimating RPE in Chapter 5 was possible by coupling field and laboratory measurements. Similarly, trenching could be coupled with Tuneable Diode Laser absorption spectrometers (TDL) that can continuously measure natural abundance of carbon isotopes both on and off-site with little

disturbance (Moyes et al. 2010, Powers et al. 2010). TDL can also be used for measuring of the isotopic composition of CO<sub>2</sub> efflux of both soil and aboveground compartments of trees after they were submitted to <sup>13</sup>C-CO<sub>2</sub> pulse labelling (Plain et al. 2009). The partitioning of components of ecosystem respiration thanks to TDL technology (Zhang et al. 2006) seems a useful approach to separating autotrophic and heterotrophic soil respiration, as well as helping account for the root priming effect.

### ***7.2 Autotroph-heterotroph continuum***

Because of the uncertainty remaining in separating autotrophic respiration from heterotrophic respiration, Hogberg et al. (2005) suggested an autotroph-heterotroph continuum (see Chapter 1). The existence of an autotroph-heterotroph continuum was illustrated in the unveiling of the root priming effects (RPE) in the soil of the *Eucalyptus regnans* (Mountain Ash) forest at Mt Disappointment, regenerating after being burnt less than a year prior (see Chapter 5). In these soils, microbial biomass was clearly stimulated by the presence of roots. Recent methods for separating the various component of soil respiration have led to detailed studies of root priming effects. Mostly those studies have provided assessment of the scale of the effect but little guidance as to its cause, other than general assertions about ‘co-metabolism’ and supplies of C. While there are reports of negative RPE (reducing respiration by up to 50%), most studies report strong increases in respiration (by up to 320%, Cheng and Kuzyakov 2005). The extent of the priming effect is dependent on species composition (Dijkstra and Cheng 2007a), plant phenology (Cheng et al. 2003), atmospheric [CO<sub>2</sub>] (Cardon 1996), plant biomass (Dijkstra et al. 2006), nutrient status of soil (Liljeroth et al. 1994) and soil moisture (Dijkstra and Cheng 2007b). All of these are in turn predicted to be influenced by climate change (Solomon et al.

2007). RPE provides a link between changes in atmospheric conditions, plants and soil respiration of heterotrophic origin. For instance, in a given ecosystem, warmer air temperatures may cause a change in plant phenology or a shift in plant species (Walther et al. 2002) but may not translate into warmer soil temperatures to an extent that soil microbe activity and/or turnover are significantly enhanced. However, and through its impact on above ground vegetation and RPE, climate warming seems likely to affect on heterotrophic respiration.

Similarly, heterotrophic soil respiration is stimulated by increased concentrations of atmospheric CO<sub>2</sub>, mostly through greater root growth and turnover, as well as enhanced production of litter, leading to an increase in the amounts of organic substrate available for heterotrophs (Deng et al. 2010, Zak et al. 2000). Increases in root production are accompanied by enhanced production and turnover of root exudates that are the main contributors to the increase in heterotrophic respiration (Heath et al. 2005). Root exudates also contribute in enhancing decomposition of recalcitrant soil organic matter (SOM) by free-living microbes (Trueman and Gonzalez-Meler 2005), hence increasing the impact of elevated atmospheric [CO<sub>2</sub>] on soil respiration through the mechanism of priming effect.

Chapter 6 showed that increased inputs of aboveground litter contributed to increased heterotrophic respiration from soil of the Australian high country. The litter addition induced a priming effect that led to the release of between 26% and 170% more CO<sub>2</sub> from these soils than would have been emitted by the unamended soils and the sole decomposing litter depending on nature of the soils and amount and origin of added litter. Litter amendment in the field however, did not deliver any changes in soil respiration which shows that there is a delay between production of litter and observing the actual alterations it may have on carbon cycling. Green litter has faster

rates of decay than abscised litter such as that used in the study in Chapter 6 (Woods and Raison 1983). Litter of Snow Gums (*E. Pauciflora*) can show a 25% weight loss for the first 12 months of decomposition, whilst other subalpine Eucalypt species such as Alpine Ash (*E. delegatensis*) can lose up to 39% of their weight (Woods and Raison 1983). By comparison, in tropical climates, litter of *E. urograndis* lost 20-30% of its dry mass over the first three months of decomposition (Ngao et al. 2009). Moreover, a short term increase in belowground respiration is sometimes observed in the weeks following application of litter to soils possibly due to a flush of easily decomposable compounds washed away from the fresh litter (Subke et al. 2004). This suggests temporal variability in decomposition of litter from *Eucalyptus sp.* The impact of a climate-fuelled increase of litter production on heterotrophic respiration may thus vary seasonally or could be affected by unusual increases in litter input such as after extreme weather events which occurrence is predicted to increase with climate change. Globally, litter produced by plants growing in an atmosphere enriched in CO<sub>2</sub> may be richer in lignin but poorer in N but it is still debated whether this will reduce rates of litter decomposition (Henry et al. 2005, Knops et al. 2007) and therefore rates of heterotrophic respiration.

### ***7.3 Regional specificity***

Rates of soil respiration measured in various ecosystems of south east Australia and presented in the previous chapters are faster than what is usually reported globally (Table 7.1).

Table 7.1.

Rates of soil respiration measured around the world in various forest ecosystems (in  $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ )

Forest type	Location	Respiration rates in $\text{mg m}^{-2} \text{ h}^{-1}$	References
Lodgepole pine+silver birch	UK	169-219	Heinemeyer et al. 2007
<i>Pinus sylvestris</i> + <i>Picea abies</i>	Sweden	270-580	Hokberg and Ekblad 1996
Loblolly pine (16 yo)	Nth Carolina	146	Matamala and Schlessinger 2000
Norway spruce	Nth Sweden	221-295	Comsted et al. 2006
<i>Pinus sylvestris</i>	Finland	170-220	Niinisto et al. 2004
<i>Pinus sylvestris</i>	Finland	23-167	Pajari 1995
Evergreen forest	Brazil	123-147	Goreau and Mello 1988
Maple forest	Ontario	355	Ellis 1974
Pine forest		332	
Young forest	Malaysia	106	Ceulemans et al. 1987
Poplar	Alaska	142	Ruess et al. 1996
White spruce		136	
Birch-aspen		165	
White spruce		130	
Deciduous forest	Maine	302	Fernandez et al. 1993
Conifer forest		302	
<i>Eucalyptus saligna</i> plantation	Australia	175-548	Chapter 2
<i>Eucalyptus pauciflora</i> woodland		189-945	Chapter 4
Messmate 1: <i>E. obliqua</i> , <i>E. radiata</i>		304-879	Chapter 4
Messmate 2: <i>E. obliqua</i> , <i>E. radiata</i> , <i>E. dives</i>		226-768	Chapter 4
Mountain Ash: <i>E. regnans</i>		137-872	Chapter 4



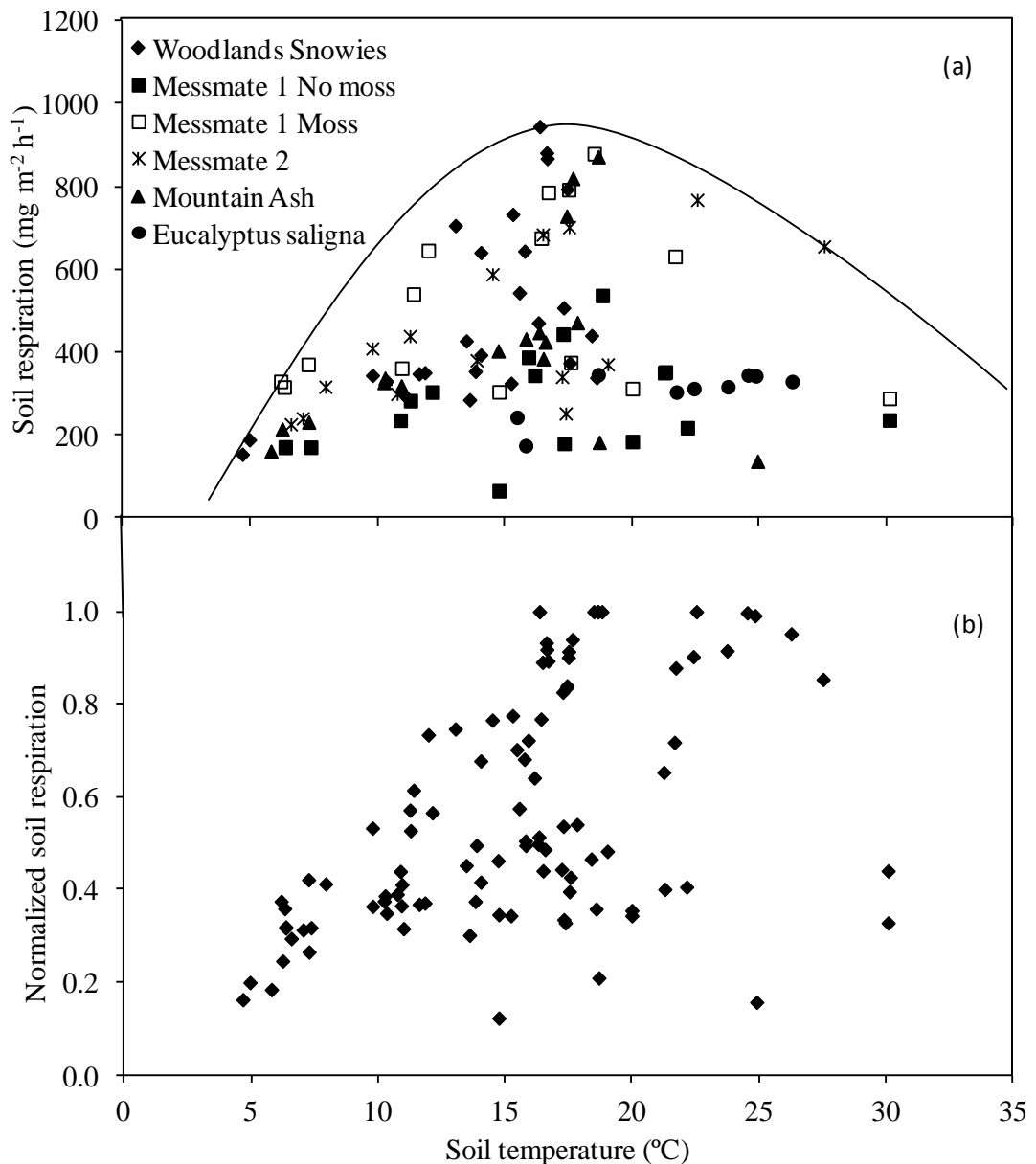


Figure 7.1.

Temperature dependence of soil respiration across all experimental sites presented in previous chapter: raw soil respiration data (a) and normalized data (relative to the maximum respiration rates observed at each site) (b). The curve drawn in (a) is not fitted to the data.

In south east Australia, soil respiration as a function of soil temperatures followed the global trend. Respiration rates increased with temperature and reached a maximum between 16 and 25°C, depending on species and locations (Figure 7.1). Above these temperatures, respiration rates slowed. The maximum soil temperatures

at which soil respiration rates were the fastest was lower than that usually observed in biochemical and physiological studies for which 45 to 50°C delivers the fastest rates (Luo and Zhou 2006). Soil microbial respiration has also been previously demonstrated to peak at 23°C (Flanagan and Veum 1974).

Temperature sensitivity of soil respiration is usually described by a  $Q_{10}$  value of approximately 2 (Chapter 1, Atkin et al. 2000, Larigauderie and Korner 1995).  $Q_{10}$  is calculated from an exponential or Arrhenius model that assumes the temperature response of soil respiration increases continuously (Bekku et al. 2004, Curiel Yuste et al. 2004, Gaumont-Guay et al. 2006, Janssens and Pilegaard 2003, Lloyd and Taylor 1994, Yuste et al. 2003, Zimmermann and Bird 2012) and does not allow respiration to decrease at greater temperatures (Khomik et al. 2009). However, soil respiration is dominated by biological processes rather than physical or chemical processes and, as was shown in the present thesis (Figure 7.1), there is an upper limit of temperature to the increase of respiration rates that may be caused by enzyme degradation (Atkin and Tjoelker 2003, Davidson and Janssens 2006).

Other factors limit the temperature response of soil respiration. The combination of microbial response, specific root respiration and root growth ensure that the temperature sensitivity of soil respiration is highly seasonal and dependent on species composition, plant phenology, stage of development and soil moisture (Luo and Zhou 2006). Experimental sites used in the studies reported in this thesis were scattered over south east Australia from near sea level to sub-alpine country and were covered with a variety of *Eucalyptus* species of varying ages. Soil respiration of eucalypts-dominated forests and woodlands of south east Australia was strongly regulated by a combination of soil temperature and soil moisture (linear model,  $P < 0.01$ ,  $n = 99$ , adjusted  $R^2 = 0.442$ ):

$$SR = 25.888 T + 12.396 VWC - 317.615$$

Where SR is soil respiration, T is temperature and VWC is volumetric water content.

Contrary to most results published in the literature that show an acceleration of respiration rates caused by elevated atmospheric [CO<sub>2</sub>] (Bernhardt et al. 2006, Deng et al. 2010, Janssens et al. 1998, Lin et al. 2001, Pataki et al. 2003, Pregitzer et al. 2006, Trueman and Gonzalez-Meler 2005, Wan et al. 2007), belowground respiration of soils supporting *Eucalyptus saligna* at the Hawkesbury Forest Experiment (HFE) was not responsive to atmospheric enrichment in CO<sub>2</sub> (see Chapter 2). The majority of studies have been conducted in the northern hemisphere and on local species. The ecology of Australian species is very specific and might not be shared by species indigenous to other continents. For instance many Australian trees are evergreen whilst being angiosperms. Moreover, the two genera *Eucalyptus* and *Acacia* dominate Australian forests and are strongly indigenous (Attiwill and Leeper 1987). Australian tree species are ancient with a long evolutionary period that can be traced from the mid-Tertiary, including periods of higher and lower atmospheric [CO<sub>2</sub>], higher and lower temperatures and higher and lower rainfall than present. This may give them the ability to better sustain environmental changes. Results obtained at the HFE, seemingly contradictory to results from experiments conducted elsewhere, illustrate the need for further research in lesser visited and researched locations and species.

In contrast to its response to elevated atmospheric [CO<sub>2</sub>] and although eucalypts have evolved for millions of years and adapted to the mostly dry Australian climate, the response of soil respiration to drought and rewatering at the HFE presented a similar

pattern to that of soils supporting species that grow elsewhere in the world. Drought significantly slowed rates of soil respiration by reducing the velocity of newly photosynthesized C travelling through the trees, soils and back to the atmosphere (Chapter 3). Rewatering of droughted soils generated a rapid increase in soil respiration. The response of soil respiration to rewatering of droughted *Eucalyptus saligna* was however short lasting and its amplitude was subdued compared to that of other soils and vegetation types. Indeed, it appears that breaking the drought at the HFE would not have incurred a significant increase in respired CO<sub>2</sub> on the long term. The adaptation of eucalypts to a dry climate must be accompanied by a similar adaptation of mycorrhizas and free living soil microbes. Because mycorrhizas are associated with roots and trees depend partly on microorganisms for nutrient supply whilst microorganisms depend on trees allocation of recently photosynthesized C, one must present similar adaptation to environmental conditions as the other does. Equally, free-living micro-organisms and macro-organisms rely on plant litter and inherent soil organic matter for survival and as such must be specialised in oxidising products of particular plants.

#### ***7.4 Fire and Australian ecosystems***

Impacts of fire as a natural recurring event has been well studied (see Chapter 1). However, this thesis considered the impact of large scale wild fires that fit the definition for mega-fire: the 0.1% of fires that account for  $\pm 95\%$  of total area burnt and  $\pm 85\%$  of total costs of fire suppression (Attiwill and Adams 2012). Mega-fires may be promoted by changes in climate. This thesis also focused on soil respiration in Australia where fires are an integrated part of the ecosystems and mega-fires have been numerous in recent years (Table 7.2). The fire-proneness, extent of the burnt

areas and intensity of fires in Australia are unique to the continent (Attiwill and Adams 2012). Combined with the specific diversity of forest stands, it makes for an intricate pattern of areas with variable rates of belowground respiration as was illustrated at the Mt Disappointment forest. Chapter 4 concluded that fire can both increase dramatically and sharpen the spatial variation in soil respiration rates at a given site. There was a clear and significant difference in respiration rates from moss-covered soils and bare soils at the Messmate 1 site of the Mount Disappointment forest. Moss beds were originally very clearly defined and soil respiration rates were faster from moss beds than from bare soils. Although the moss beds expanded and the understorey vegetation grew back during the 18 months the Mt Disappointment sites were visited, the difference in respiration rates did not disappear. Moss beds at Messmate 1 were an indicator of the conditions of soil supporting them and these conditions, that enhanced microbial activity, persisted in time. In the contrary, soil respiration under Mountain Ash stands only distant by a few kilometres, was homogeneous.

At a wider scale, the fast recovery of ‘resprouters’ trees at the Messmate 1 site at the Mt Disappointment forest (Chapter 4) was accompanied with a return to the seasonal pattern of soil respiration observed amongst ecosystems around the world. The Mountain Ash site, dominated by trees killed by fire and regenerating through germination of seeds, was still subject to sudden increases of soil respiration two years after fire. On the other hand, woodlands at the Snowy Plains showed that soil respiration on burnt plots was back to similar rates to unburnt plots within three years despite the understorey having changed to grass whereas unburnt plots presented a shrubby understorey. It seems that fire affects soil respiration in these sub-alpine Australian ecosystems mostly in the first few months following a burn. Therefore in

order to draw the most accurate picture of soil respiration and an accurate carbon budget for Australian forests, monitoring soil respiration in the first few months following fire is crucial. Early visits to experimental sites, no later than when the very first signs of recovery appear, are necessary to detect any spatial heterogeneity in carbon cycling within and across stands as well as to estimate the time-lapse between fire disturbance and full recovery of belowground respiration. Contrary to what was observed in these sub-Alpine regions, fire impact on soil respiration of Australian tropical savannas woodland can last up to six years depending on the season (Richards et al. 2012) with soil respiration greater in un-burnt plots during the wet season.

### ***7.5 Conclusion***

The work presented in this thesis has increased the knowledge of soil respiration in forests of south-east Australia; a region and biome where studies on soil respiration are lacking. As shown here, environmental conditions provide clear constraints for soil respiration that was also resilient to fires. Accurate estimates of soil respiration at a plot scale are rendered difficult by spatial variation in respiration rates either in undisturbed stands with proximity to tree stems or following fire disturbance that increase the spatial variation. Finally, root and litter priming effects must be considered respectively when separating soil respiration into its autotrophic and heterotrophic components or when estimating the impact of climate change on soil respiration. The results of this work suggest that the effect of climate change on soil respiration should be studied under more varied conditions and across ecosystems. Non-intrusive techniques, using isotopes, are preferred. Long term experiments or experiments along an age-gradient should be favoured in the case of forest

ecosystems due to the longer lifespan of the dominating species. High spatial resolution may be needed in some cases, especially after major disturbances such as fire.

Table 7.2.

Examples of mega-fires in the world. From Attiwill and Binkley, 2013.

Year	Size (millions of ha)	Location	Biome
1987	>7	China and Russia	Asia and Boreal forest
1998	9.4	Russia	
1915	1.4		
1825	1.2	New Brunswick, Canada	North American coniferous forests, boreal, subalpine and temperate
1910	1.2	Idaho, Montana and Washington, USA	
1919	2.5	Manitoba, Canada	
1988	1.4	Wyoming, USA	
1989	2.5	Manitoba, Canada	
1851	5.0	Victoria, New South Wales and South Australia, Australia	Eucalypt forest, shrublands and grasslands
1938	1.5		
1944	1.0		
2003	1.1		
2007	1.2		
2009	0.5		
1983	5.0	Indonesia	Tropical rainforest, primary and secondary forests, forest plantations and crops
1987	2.0		
1997-98	9.5		
2008	3.6	Botswana	Grassland savanna
1999	3.8	Brazil	Tropical rainforest, primary and secondary forests, deforested areas, savanna, grasslands and crops
2003-05	0.5	Portugal	Mediterranean woodlands, pine and eucalypt plantations
2007	0.3	Greece	Mediterranean woodlands



### References

- Adams, M. A. 1984. *Nitrogen cycling and mineralization in forests of south-eastern Australia*. Ph.D. thesis, University of Melbourne.
- Adams, M. A., Cunningham, S. C. and Taranto, M. T. 2013. A critical review of the science underpinning fire management in the high altitude ecosystems of south-eastern Australia. *Forest Ecology and Management*.
- Allard, V., Newton, P. C. D., Lieffering, M., Soussana, J. F., Grieu, P. and Matthew, C. 2004. Elevated CO<sub>2</sub> effects on decomposition processes in a grazed grassland. *Global Change Biology*, 10, 1553-1564.
- Allison, I., Bindoff, N. L., Bindschadler, R. A., Cox, P. M., De Noblet, N., England, M. H., Francis, J. E., Gruber, N., Haywood, A. M., Karoly, D. J., Kaser, G., Le Quéré, C., Lenton, T. M., Mann, M. E., Mcneil, B. I., Pitman, A. J., Rahmstorf, S., Rignot, E., Schellnhuber, H. J., Schneider, S. H., Sherwood, S. C., Somerville, R. C. J., Steffen, K., Steig, E. J., Visbeck, M. and Weaver, A. J. 2009. The Copenhagen diagnosis, 2009: updating the world on the latest climate science. 60.
- Almagro, M., Lopez, J., Querejeta, J. I. and Martinez-Mena, M. 2009. Temperature dependence of soil CO<sub>2</sub> efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem. *Soil Biology & Biochemistry*, 41, 594-605.
- Andrews, J. A., Harrison, K. G., Matamala, R. and Schlesinger, W. H. 1999. Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). *Soil Sci Soc Am J*, 63, 1429-1435.
- Asensio, D., Penuelas, J., Llusia, J., Ogaya, R. and Filella, L. 2007. Interannual and interseasonal soil CO<sub>2</sub> efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. *Soil Biology & Biochemistry*, 39, 2471-2484.
- Atkin, O. K. and Tjoelker, M. G. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, 8, 343-351.
- Atkin, O. K., Edwards, E. J. and Loveys, B. R. 2000. Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist*, 147, 141-154.
- Attiwill, P. M. 1994. Ecological disturbance and the conservative management of eucalypt forests in Australia. *Forest Ecology and Management*, 63, 301-346.
- Attiwill, P. M. and Adams, M. A. 2012. Mega-fires, inquiries and politics in the eucalypt forests of Victoria, south-eastern Australia. *Forest Ecology and Management*.
- Attiwill, P. and Binkley, D. 2013. Exploring the mega-fire reality: A 'Forest Ecology and Management' conference. *Forest Ecology and Management*.
- Attiwill, P. M. and Leeper, G. W. 1987. *Forest soils and nutrient cycles*, Melbourne, Melbourne University Press.

- Bader, M. K. F. and Korner, C. 2010. No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO<sub>2</sub> enrichment. *Global Change Biology*, 16, 2830-2843.
- Bader, N. E. and Cheng, W. X. 2007. Rhizosphere priming effect of *Populus fremontii* obscures the temperature sensitivity of soil organic carbon respiration. *Soil Biology & Biochemistry*, 39, 600-606.
- Bahn, M., Schmitt, M., Siegwolf, R., Richter, A. and Bruggemann, N. 2009. Does photosynthesis affect grassland soil-respired CO<sub>2</sub> and its carbon isotope composition on a diurnal timescale? *New Phytologist*, 182, 451-460.
- Baldocchi, D., Tang, J. W. and Xu, L. K. 2006. How switches and lags in biophysical regulators affect spatial-temporal variation of soil respiration in an oak-grass savanna. *Journal of Geophysical Research-Biogeosciences*, 111, 13.
- Barcenas-Moreno, G. and Baath, E. 2009. Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. *Soil Biology & Biochemistry*, 41, 2517-2526.
- Barton, C. V. M., Ellsworth, D. S., Medlyn, B. E., Duursma, R. A., Tissue, D. T., Adams, M. A., Eamus, D., Conroy, J. P., McMurtrie, R. E., Parsby, J. and Linder, S. 2010. Whole-tree chambers for elevated atmospheric CO<sub>2</sub> experimentation and tree scale flux measurements in south-eastern Australia: The Hawkesbury Forest Experiment. *Agricultural and Forest Meteorology*, 150, 941-951.
- Bear, R. and Pickering, C. M. 2006. Recovery of subalpine grasslands from bushfire. *Australian Journal of Botany*, 54, 451-458.
- Bekku, Y. S., Nakatsubo, T., Kume, A. and Koizumi, H. 2004. Soil microbial biomass, respiration rate, and temperature dependence on a successional glacier foreland in Ny-Ålesund, Svalbard. *Arctic, Antarctic, and Alpine Research*, 36, 395-399.
- Bernhardt, E. S., Barber, J. J., Phippen, J. S., Taneva, L., Andrews, J. A. and Schlesinger, W. H. 2006. Long-term effects of free air CO<sub>2</sub> enrichment (FACE) on soil respiration. *Biogeochemistry*, 77, 91-116.
- Bhupinderpal-Singh, Nordgren, A., Lofvenius, M. O., Hogberg, M. N., Mellander, P. E. and Hogberg, P. 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell and Environment*, 26, 1287-1296.
- Bingeman, C. W., Varner, J. E. and Martin, W. P. 1953. The effect of the addition of organic materials to the decomposition of an organic soil. *Soil Science Society of America Proceedings*, 17, 34-38.
- Binkley, D., Stape, J. L., Takahashi, E. N. and Ryan, M. G. 2006. Tree-girdling to separate root and heterotrophic respiration in two Eucalyptus stands in Brazil. *Oecologia*, 148, 447-454.
- Blagodatskaya, E. and Kuzyakov, Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils*, 45, 115-131.
- Boden, T. A., Marland, G. and Andres, R. J. 2010. Global, regional, and national fossil-fuel CO<sub>2</sub> emissions. Oak Ridge, Tenn., U.S.A. : Carbon Dioxide

- Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy.
- Bond-Lamberty, B. & Thomson, A. 2010. Temperature-associated increases in the global soil respiration record. *Nature*, 464, 579-U132.
- Bond-Lamberty, B., Wang, C. K. and Gower, S. T. 2004. Contribution of root respiration to soil surface CO<sub>2</sub> flux in a boreal black spruce chronosequence. *Tree Physiology*, 24, 1387-1395.
- Boone, R. D., Nadelhoffer, K. J., Canary, J. D. and Kaye, J. P. 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, 396, 570-572.
- Borken, W., Savage, K., Davidson, E. A. and Trumbore, S. E. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*, 12, 177-193.
- Bowman, D., Balch, J. K., Artaxo, P., Bond, W. J., Carlson, J. M., Cochrane, M. A., D'antonio, C. M., Defries, R. S., Doyle, J. C., Harrison, S. P., Johnston, F. H., Keeley, J. E., Krawchuk, M. A., Kull, C. A., Marston, J. B., Moritz, M. A., Prentice, I. C., Roos, C. I., Scott, A. C., Swetnam, T. W., Van Der Werf, G. R. and Pyne, S. J. 2009. Fire in the earth system. *Science*, 324, 481-484.
- Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. E., Reynolds, J. F., Treseder, K. K. and Wallenstein, M. D. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, 11, 1316-1327.
- Bronson, D. R., Gower, S. T., Tanner, M., Linder, S. and Van Herk, I. 2008. Response of soil surface CO<sub>2</sub> flux in a boreal forest to ecosystem warming. *Global Change Biology*, 14, 856-867.
- Burke, J. M., Prepas, E. E. & Pinder, S. 2005. Runoff and phosphorus export patterns in large forested watersheds on the western Canadian boreal plain before and for 4 years after wildfire. *Journal of Environmental Engineering and Science*, 4, 319-325.
- Butnor, J. R., Johnsen, K. H., Oren, R. and Katul, G. G. 2003. Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands. *Global Change Biology*, 9, 849-861.
- Carbone, M. S. and Trumbore, S. E. 2007. Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. *New Phytologist*, 176, 124-135.
- Carbone, M. S., Czimczik, C. I., McDuffee, K. E. and Trumbore, S. E. 2007. Allocation and residence time of photosynthetic products in a boreal forest using a low-level <sup>14</sup>C pulse-chase labeling technique. *Global Change Biology*, 13, 466-477.
- Carbone, M. S., Winston, G. C. and Trumbore, S. E. 2008. Soil respiration in perennial grass and shrub ecosystems: Linking environmental controls with plant and microbial sources on seasonal and diel timescales. *Journal of Geophysical Research-Biogeosciences*, 113, 14.

- Cardon, Z. G. 1996. Influence of rhizodeposition under elevated CO<sub>2</sub> on plant nutrition and soil organic matter. *Plant and Soil*, 187, 277-288.
- Ceulemans, R., Impens, I. and Gabriéls, R. 1987. CO<sub>2</sub> evolution from different types of soil cover in the tropics. *Tropical Agriculture*, 64, 68-69
- Cheng, W. X. 2009. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biology & Biochemistry*, 41, 1795-1801.
- Cheng, W. X., Johnson, D. W. and Fu, S. L. 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology, and fertilization. *Soil Science Society of America Journal*, 67, 1418-1427.
- Cheng, W. X. and Kuzyakov, Y. 2005. Root effects on soil organic matter decomposition. In: Zobel R.W. and Wright S.F. (eds.) *Roots and soil management: interactions between roots and the soil*. Madison, Wisconsin: ASA-SSSA.
- Cisneros-Dozal, L. M., Trumbore, S. and Hanson, P. J. 2006. Partitioning sources of soil-respired CO<sub>2</sub> and their seasonal variation using a unique radiocarbon tracer. *Global Change Biology*, 12, 194-204.
- Comstedt, D., Bostrom, B., Marshall, J. D., Holm, A., Slaney, M., Linder, S. and Ekblad, A. 2006. Effects of elevated atmospheric carbon dioxide and temperature on soil respiration in a boreal forest using  $\delta^{13}\text{C}$  as a labeling tool. *Ecosystems*, 9, 1266-1277.
- Cox, P. M., Betts, R. A., Jones, C. D., Spall, S. A. and Totterdell, I. J. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, 408, 184-187.
- Crow, S. E., Lajtha, K., Bowden, R. D., Yano, Y., Brant, J. B., Caldwell, B. A. and Sulzman, E. W. 2009. Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. *Forest Ecology and Management*, 258, 2224-2232.
- CSIRO and BOM 2007. Climate change in Australia-Technical report 2007. 148.
- Curiel Yuste, J., Janssens, I. A., Carrara, A. and Ceulemans, R. 2004. Annual Q<sub>10</sub> of soil respiration reflects plant phenological patterns as well as temperature sensitivity. *Global Change Biology*, 10, 161-169.
- Czimczik, C. I., Trumbore, S. E., Carbone, M. S. and Winston, G. C. 2006. Changing sources of soil respiration with time since fire in a boreal forest. *Global Change Biology*, 12, 957-971.
- Davidson, E. A. and Janssens, I. A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440, 165-173.
- Davidson, E. A., Janssens, I. A. and Luo, Y. Q. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q<sub>10</sub>. *Global Change Biology*, 12, 154-164.
- Davidson, E. A., Nepstad, D. C., Ishida, F. Y. and Brando, P. M. 2008. Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, 14, 2582-2590.

- Debano, L. F. 2000. The role of fire and soil heating on water repellency in wildland environments: a review. *Journal of Hydrology*, 231, 195-206.
- Deforest, J. L., Noormets, A., McNulty, S. G., Sun, G., Tenney, G. and Chen, J. Q. 2006. Phenophases alter the soil respiration-temperature relationship in an oak-dominated forest. *International Journal of Biometeorology*, 51, 135-144.
- Deng, Q., Zhou, G., Liu, J., Liu, S., Duan, H. and Zhang, D. 2010. Responses of soil respiration to elevated carbon dioxide and nitrogen addition in young subtropical forest ecosystems in China. *Biogeosciences*, 7, 315-328.
- De Nobili, M., Contin, M., Mondini, C. and Brookes, P. C. 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology & Biochemistry*, 33, 1163-1170.
- Diaz-Pines, E., Schindlbacher, A., Pfeffer, M., Jandl, R., Zechmeister-Boltenstern, S. and Rubio, A. 2010. Root trenching: a useful tool to estimate autotrophic soil respiration? A case study in an Austrian mountain forest. *European Journal of Forest Research*, 129, 101-109.
- Dijkstra, F. A. and Cheng, W. X. 2007a. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecology Letters*, 10, 1046-1053.
- Dijkstra, F. A. and Cheng, W. X. 2007b. Moisture modulates rhizosphere effects on C decomposition in two different soil types. *Soil Biology & Biochemistry*, 39, 2264-2274.
- Dijkstra, F. A., Cheng, W. X. and Johnson, D. W. 2006. Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. *Soil Biology & Biochemistry*, 38, 2519-2526.
- Dilly, O. 2001. Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter. *Soil Biology & Biochemistry*, 33, 117-127.
- Dilly, O. 2003. Regulation of the respiratory quotient of soil microbiota by availability of nutrients. *Fems Microbiology Ecology*, 43, 375-381.
- Drake, B. G., Leadley, P. W., Arp, W. J., Nassiry, D. and Curtis, P. S. 1989. An open top chamber for field studies of elevated atmospheric CO<sub>2</sub> concentration on saltmarsh vegetation. *Functional Ecology*, 3, 363-371.
- Dumas, S., Neufeld, H. S. and Fisk, M. C. 2007. Fire in a thermic oak-pine forest in Linville Gorge Wilderness Area, North Carolina: Importance of the shrub layer to ecosystem response. *Castanea*, 72, 92-104.
- Duursma, R. A., Barton, C. V., Eamus, D., Medlyn, B. E., Ellsworth, D. S., Forster, M. A., Tissue, D. T., Linder, S. and Mcmurtrie, R. E. 2011. Rooting depth explains [CO<sub>2</sub>]× drought interaction in *Eucalyptus saligna*. *Tree Physiology*, 31, 922-931.
- Edwards, N. T. and Norby, R. J. 1999. Below-ground respiratory responses of sugar maple and red maple saplings to atmospheric CO<sub>2</sub> enrichment and elevated air temperature. *Plant and Soil*, 206, 85-97.

- Ekblad, A. and Högberg, P. 2001. Natural abundance of  $^{13}\text{C}$  in  $\text{CO}_2$  respired from forest soils reveals speed of link between tree photosynthesis and root respiration. *Oecologia*, 127, 305-308.
- Ekblad, A., Boström, B., Holm, A. and Comstedt, D. 2005. Forest soil respiration rate and  $\delta^{13}\text{C}$  is regulated by recent above ground weather conditions. *Oecologia*, 143, 136-142.
- Eliasson, P. E., Mcmurtrie, R. E., Pepper, D. A., Stromgren, M., Linder, S. and Agren, G. I. 2005. The response of heterotrophic  $\text{CO}_2$  flux to soil warming. *Global Change Biology*, 11, 167-181.
- Ellis, R. C. 1974. The seasonal pattern of nitrogen and carbon mineralization in forests and pasture soils in southern Ontario. *Canadian Journal of Soil Science*, 54, 15-28.
- Epron, D., Farque, L., Lucot, E. and Badot, P. M. 1999. Soil  $\text{CO}_2$  efflux in a beech forest: the contribution of root respiration. *Annals of Forest Science*, 56, 289-295.
- Epron, D., Le Dantec, V., Dufrene, E. and Granier, A. 2001. Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. *Tree Physiology*, 21, 145-152.
- Falge, E., Baldocchi, D., Tenhunen, J., Aubinet, M., Bakwin, P., Berbigier, P., Bernhofer, C., Burba, G., Clement, R., Davis, K. J., Elbers, J. A., Goldstein, A. H., Grelle, A., Granier, A., Guomundsson, J., Hollinger, D., Kowalski, A. S., Katul, G., Law, B. E., Malhi, Y., Meyers, T., Monson, R. K., Munger, J. W., Oechel, W., Paw, K. T., Pilegaard, K., Rannik, U., Rebmann, C., Suyker, A., Valentini, R., Wilson, K. and Wofsy, S. 2002. Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. *Agricultural and Forest Meteorology*, 113, 53-74.
- Fang, C. and Moncrieff, J. B. 2001. The dependence of soil  $\text{CO}_2$  efflux on temperature. *Soil Biology & Biochemistry*, 33, 155-165.
- Fernandez, D. P., Neff, J. C., Belnap, J. and Reynolds, R. L. 2006. Soil respiration in the cold desert environment of the Colorado Plateau (USA): Abiotic regulators and thresholds. *Biogeochemistry*, 78, 247-265.
- Fernandez, I. J., Son, Y., Kraske, C. R., Rustad, L. E. and David, M. B. 1993. Soil carbon dioxide characteristics under different forest types and after harvest. *Soil Science Society of America Journal*, 57, 1115-1121.
- Field, C. B., Jackson, R. B. and Mooney, H. A. 1995. Stomatal responses to increased  $\text{CO}_2$  - Implications from the plant to the global-scale. *Plant Cell and Environment*, 18, 1214-1225.
- Flanagan, P. W. and Veum, A. K. 1974. Relationships between respiration, weight loss, temperature and moisture in organic residues on tundra. *In: Holding, A. J., Heal, O. W., Maclean, S. F. J. and Flanagan, P. W. (eds.) Soil organisms and decomposition in tundra*. Stockholm, Sweden: IBP Tundra Biome Steering Committee.
- Flannigan, M. D. and Vanwagner, C. E. 1991. Climate change and wildfire in Canada. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, 21, 66-72.

- Fog, K. 1988. The effect of added Nitrogen on the rate of decomposition of organic-matter. *Biological Reviews of the Cambridge Philosophical Society*, 63, 433-462.
- Fontaine, S., Bardoux, G., Abbadie, L. and Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. *Ecology Letters*, 7, 314-320.
- Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., Mary, B., Revaillet, S. and Maron, P. A. 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry*, 43, 86-96.
- Fontaine, S., Mariotti, A. and Abbadie, L. 2003. The priming effect of organic matter: a question of microbial competition? *Soil Biology & Biochemistry*, 35, 837-843.
- Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., Haywood, J., Lean, J., Lowe, D. C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M. and Dorland, A. R. V. 2007. Changes in Atmospheric Constituents and in Radiative Forcing. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H. L. (eds.) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.
- Frey, B., Hagedorn, F. and Giudici, F. 2006. Effect of girdling on soil respiration and root composition in a sweet chestnut forest. *Forest Ecology and Management*, 225, 271-277.
- Garten, C. T., Classen, A. T. and Norby, R. J. 2009. Soil moisture surpasses elevated CO<sub>2</sub> and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment. *Plant and Soil*, 319, 85-94.
- Gaudinski, J. B., Trumbore, S. E., Davidson, E. A. and Zheng, S. H. 2000. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry*, 51, 33-69.
- Gaumont-Guay, D., Black, T. A., Griffis, T. J., Barr, A. G., Morgenstern, K., Jassal, R. S. and Nesic, Z. 2006. Influence of temperature and drought on seasonal and interannual variations of soil, bole and ecosystem respiration in a boreal aspen stand. *Agricultural and Forest Meteorology*, 140, 203-219.
- Giardina, C. P., Binkley, D., Ryan, M. G., Fownes, J. H. and Senock, R. S. 2004. Belowground carbon cycling in a humid tropical forest decreases with fertilization. *Oecologia*, 139, 545-550.
- Goreau, T. J. and Mello, W. Z. D. 1988. Tropical deforestation: some effects on atmospheric chemistry. *Ambio*, 17, 275-281.
- Gough, C. M., Vogel, C. S., Harrold, K. H., George, K. and Curtis, P. S. 2007. The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. *Global Change Biology*, 13, 1935-1949.
- Hamer, U. and Marschner, B. 2005. Priming effects in soils after combined and repeated substrate additions. *Geoderma*, 128, 38-51.

- Hamilton, J. G., Dalucia, E. H., George, K., Naidu, S. L., Finzi, A. C. and Schlessinger, W. H. 2002. Forest carbon balance under elevated CO<sub>2</sub>. *Oecologia*, 131, 250-260.
- Hamman, S. T., Burke, I. C. and Stromberger, M. E. 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. *Soil Biology & Biochemistry*, 39, 1703-1711.
- Hanson, P. J., Edwards, N. T., Garten, C. T. and Andrews, J. A. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry*, 48, 115-146.
- Harper, C. W., Blair, J. M., Fay, P. A., Knapp, A. K. and Carlisle, J. D. 2005. Increased rainfall variability and reduced rainfall amount decreases soil CO<sub>2</sub> flux in a grassland ecosystem. *Global Change Biology*, 11, 322-334.
- Hartley, I. P., Heinemeyer, A. and Ineson, P. 2007. Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, 13, 1761-1770.
- Hartley, I. P., Hopkins, D. W., Garnett, M. H., Sommerkorn, M. and Wookey, P. A. 2008. Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecology Letters*, 11, 1092-1100.
- Hasson, A. E. A., Mills, G. A., Timbal, B. and Walsh, K. 2009. Assessing the impact of climate change on extreme fire weather events over southeastern Australia. *Climate Research*, 39, 159-172.
- Hawkes, C. V., Hartley, I. P., Ineson, P. and Fitter, A. H. 2008. Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. *Global Change Biology*, 14, 1181-1190.
- Heath, J., Ayres, E., Possell, M., Bardgett, R. D., Black, H. I. J., Grant, H., Ineson, P. and Kerstiens, G. 2005. Rising atmospheric CO<sub>2</sub> reduces sequestration of root-derived soil carbon. *Science*, 309, 1711-1713.
- Heimann, M. and Reichstein, M. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature*, 451, 289-292.
- Heinemeyer, A., Hartley, I. P., Evans, S. P., Carreira De La Fuente, J. A. and Ineson, P. 2007. Forest soil CO<sub>2</sub> flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, 13, 1786-1797.
- Hendrey, G. R., Ellsworth, D. S., Lewin, K. F. and Nagy, J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO<sub>2</sub>. *Global Change Biology*, 5, 293-309.
- Henry, H. a. L., Cleland, E. E., Field, C. B. and Vitousek, P. M. 2005. Interactive effects of elevated CO<sub>2</sub>, N deposition and climate change on plant litter quality in a California annual grassland. *Oecologia*, 142, 465-473.
- Hogberg, P., Bhupinderpal, S., Lofvenius, M. O. and Nordgren, A. 2009. Partitioning of soil respiration into its autotrophic and heterotrophic components by means of tree-girdling in old boreal spruce forest. *Forest Ecology and Management*, 257, 1764-1767.



- Hogberg, P. and Ekblad, A. 1996. Substrate-induced respiration measured in situ in a C<sub>3</sub>-plant ecosystem using additions of C<sub>4</sub>-sucrose. *Soil Biology & Biochemistry*, 28, 1131-1138.
- Hogberg, P., Hogberg, M. N., Gottlicher, S. G., Betson, N. R., Keel, S. G., Metcalfe, D. B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S. and Nasholm, T. 2008. High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. *New Phytologist*, 177, 220-228.
- Högberg, P., Nordgren, A. and Ågren, G. 2002. Carbon allocation between tree root growth and root respiration in boreal pine forest. *Oecologia*, 132, 579-581.
- Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A. F. S., Ekblad, A., Hogberg, M. N., Nyberg, G., Ottosson-Lofvenius, M. and Read, D. J. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, 411, 789-792.
- Högberg, P., Nordgren, A., Högberg, M. N., Ottosson-Löfvenius, M., Bhupinderpal-Singh, Olsson, P. and Linder, S. 2005. Fractional contributions by autotrophic and heterotrophic respiration to soil-surface CO<sub>2</sub> efflux in boreal forests. In: Griffiths, H. and Jarvis, P. G. (eds.) *The Carbon Balance of Forest Biomes*. Oxford: Taylor & Francis.
- Hoosbeek, M. R., Vos, J. M., Meinders, M. B. J., Velthorst, E. J. and Scarascia-Mugnozza, G. E. 2007. Free atmospheric CO<sub>2</sub> enrichment (FACE) increased respiration and humification in the mineral soil of a poplar plantation. *Geoderma*, 138, 204-212.
- Horwath, W. R., Pregitzer, K. S. and Paul, E. A. 1994. <sup>14</sup>C allocation in tree-soil systems. *Tree Physiology*, 14, 1163-1176.
- Hungate, B. A., Holland, E. A., Jackson, R. B., Chapin, F. S., Mooney, H. A. and Field, C. B. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, 388, 576-579.
- Huxman, T. E., Snyder, K. A., Tissue, D., Leffler, A. J., Ogle, K., Pockman, W. T., Sandquist, D. R., Potts, D. L. and Schwinning, S. 2004. Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. *Oecologia*, 141, 254-268.
- Irvine, J., Law, B. E. and Hibbard, K. A. 2007. Postfire carbon pools and fluxes in semiarid ponderosa pine in Central Oregon. *Global Change Biology*, 13, 1748-1760.
- Iversen, C. M., Ledford, J. and Norby, R. J. 2008. CO<sub>2</sub> enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytologist*, 179, 837-847.
- Jackson, R. B., Banner, J. L., Jobbagy, E. G., Pockman, W. T. and Wall, D. H. 2002. Ecosystem carbon loss with woody plant invasion of grasslands. *Nature*, 418, 623-626.
- Janssens, I. A., Crookshanks, M., Taylor, G. and Ceulemans, R. 1998. Elevated atmospheric CO<sub>2</sub> increases fine root production, respiration, rhizosphere respiration and soil CO<sub>2</sub> efflux in Scots pine seedlings. *Global Change Biology*, 4, 871-878.

- Janssens, I. A., Dieleman, W., Luysaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E. D., Tang, J. and Law, B. E. 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, 3, 315-322.
- Janssens, I. A., Lankreijer, H., Matteucci, G., Kowalski, A. S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E. J., Grelle, A., Rannik, U., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N. O., Vesala, T., Granier, A., Schulze, E. D., Lindroth, A., Dolman, A. J., Jarvis, P. G., Ceulemans, R. and Valentini, R. 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, 7, 269-278.
- Janssens, I. A. and Pilegaard, K. I. M. 2003. Large seasonal changes in  $Q_{10}$  of soil respiration in a beech forest. *Global Change Biology*, 9, 911-918.
- Jarvis, P., Rey, A., Petsikos, C., Wingate, L., Rayment, M., Pereira, J., Banza, J., David, J., Miglietta, F., Borghetti, M., Manca, G. and Valentini, R. 2007. Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: the "Birch effect". *Tree Physiology*, 27, 929-940.
- Jassal, R. S. and Black, T. A. 2006. Estimating heterotrophic and autotrophic soil respiration using small-area trenched plot technique: Theory and practice. *Agricultural and Forest Meteorology*, 140, 193-202.
- Jassal, R. S., Black, T. A., Novak, M. D., Gaumont-Guay, D. and Nesic, Z. 2008. Effect of soil water stress on soil respiration and its temperature sensitivity in an 18-year-old temperate Douglas-fir stand. *Global Change Biology*, 14, 1305-1318.
- Jastrow, J. D., Michael Miller, R., Matamala, R., Norby, R. J., Boutton, T. W., Rice, C. W. and Owensby, C. E. 2005. Elevated atmospheric carbon dioxide increases soil carbon. *Global Change Biology*, 11, 2057-2064.
- Jenkins, M. and Adams, M. A. 2010. Vegetation type determines heterotrophic respiration in subalpine Australian ecosystems. *Global Change Biology*, 16, 209-219.
- Jenkins, M. E. and Adams, M. A. 2011. Respiratory quotients and  $Q_{10}$  of soil respiration in sub-alpine Australia reflect influences of vegetation types. *Soil Biology & Biochemistry*, 43, 1266-1274.
- Johnson, D., Leake, J. R., Ostle, N., Ineson, P. and Read, D. J. 2002. In situ  $^{13}\text{CO}_2$  pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, 153, 327-334.
- Jones, D. L. 1998. Organic acids in the rhizosphere - a critical review. *Plant and Soil*, 205, 25-44.
- Kara, O. and Bolat, I. 2009. Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. *Ecological Indicators*, 9, 1151-1155.

- Kasurinen, A., Kokko-Gonzales, P., Riikonen, J., Vapaavuori, E. and Holopainen, T. 2004. Soil CO<sub>2</sub> efflux of two silver birch clones exposed to elevated CO<sub>2</sub> and O<sub>3</sub> levels during three growing seasons. *Global Change Biology*, 10, 1654-1665.
- Kayler, Z., Gessler, A. and Buchmann, N. 2010. What is the speed of link between aboveground and belowground processes? *New Phytologist*, 187, 885-888.
- Keith, H., Jacobsen, K. L. and Raison, R. J. 1997. Effects of soil phosphorus availability, temperature and moisture on soil respiration in *Eucalyptus pauciflora* forest. *Plant and Soil*, 190, 127-141.
- Kellomaki, S., Wang, K. Y. and Lemettinen, M. 2000. Controlled environment chambers for investigating tree response to elevated CO<sub>2</sub> and temperature under boreal conditions. *Photosynthetica*, 38, 69-81.
- Khanna, P. K. and Raison, R. J. 1986. Effect of fire intensity on solution chemistry of surface soil under a *Eucalyptus pauciflora* forest. *Australian Journal of Soil Research*, 24, 423-434.
- Khomik, M., Arain, M. A., Liaw, K.-L. and Mccaughey, J. H. 2009. Debut of a flexible model for simulating soil respiration–soil temperature relationship: Gamma model. *Journal of Geophysical Research: Biogeosciences*, 114, G03004.
- King, J. S., Hanson, P. J., Bernhardt, E., Deangelis, P., Norby, R. J. and Pregitzer, K. S. 2004. A multiyear synthesis of soil respiration responses to elevated atmospheric CO<sub>2</sub> from four forest FACE experiments. *Global Change Biology*, 10, 1027-1042.
- Kirschbaum, M. U. F. 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, 10, 1870-1877.
- Knops, J. M. H., Naeemw, S. and Reich, P. B. 2007. The impact of elevated CO<sub>2</sub>, increased nitrogen availability and biodiversity on plant tissue quality and decomposition. *Global Change Biology*, 13, 1960-1971.
- Knorr, M., Frey, S. D. and Curtis, P. S. 2005. Nitrogen additions and litter decomposition: A meta-analysis. *Ecology*, 86, 3252-3257.
- Kutiel, P. and Naveh, Z. 1987. The effect of fire on nutrients in a pine forest soil. *Plant and Soil*, 104, 269-274.
- Kuzyakov, Y. and Cheng, W. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology & Biochemistry*, 33, 1915-1925.
- Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, 165, 382-396.
- Kuzyakov, Y. 2006. Sources of CO<sub>2</sub> efflux from soil and review of partitioning methods. *Soil Biology & Biochemistry*, 38, 425-448.
- Kuzyakov, Y., Friedel, J. K. and Stahr, K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry*, 32, 1485-1498.

- Kuzyakov, Y. and Gavrichkova, O. 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. *Global Change Biology*, 16.
- Kuzyakov, Y. and Larionova, A. A. 2005. Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, 168, 503-520.
- Lagerstrom, A., Esberg, C., Wardle, D. A. and Giesler, R. 2009. Soil phosphorus and microbial response to a long-term wildfire chronosequence in northern Sweden. *Biogeochemistry*, 95, 199-213.
- Larigauderie, A. and Korner, C. 1995. Acclimation of leaf dark respiration to temperature in alpine and lowland plant-species. *Annals of Botany*, 76, 245-252.
- Leadley, P. W. and Drake, B. G. 1993. Open top chamber for exposing plant canopy to elevated CO<sub>2</sub> concentration and for measuring net gas-exchange *Vegetatio*, 104, 3-15.
- Lewin, K. F., Hendrey, G. R., Nagy, J. and Lamorte, R. L. 1994. Design and application of a free air carbon-dioxide enrichment facility. *Agricultural and Forest Meteorology*, 70, 15-29.
- Liljeroth, E., Kuikman, P. and Vanveen, J. A. 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic-matter at different soil-nitrogen levels. *Plant and Soil*, 161, 233-240.
- Lin, G., Rygielwicz, P. T., Ehleringer, J. R., Johnson, M. G. and Tingey, D. T. 2001. Time-dependent responses of soil CO<sub>2</sub> efflux components to elevated atmospheric [CO<sub>2</sub>] and temperature in experimental forest mesocosms. *Plant and Soil*, 229, 259-270.
- Lloyd, J. and Taylor, J. A. 1994. On the temperature dependence of soil respiration. *Functional Ecology*, 8, 315-323.
- Loiseau, P. and Soussana, J. F. 1999. Elevated [CO<sub>2</sub>], temperature increase and N supply effects on the turnover of below-ground carbon in a temperate grassland ecosystem. *Plant and Soil*, 210, 233-247.
- Luo, Y., Gerten, D., Le Maire, G., Parton, W. J., Keough, C., Weng, E., Beier, C., Ciais, P., Cramer, W., Dukes, J. S., Hanson, P. J., Knapp, A. a. K., Linder, S., Nepstad, D., Rustad, L. and Sowerby, A. 2008. Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology*, 14, 2365-2379.
- Luo, Y. Q., Wan, S. Q., Hui, D. F. and Wallace, L. L. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, 413, 622-625.
- Luo, Y. and Zhou, X. 2006. *Soil respiration and the environment*, London San Diego, Elsevier.
- Mandl, R. H., Weinstein, L. H., Mccune, D. C. and Keveny, M. 1973. A cylindrical, open-top chamber for the exposure of plants to air pollutants in the field. *J Environ Qual*, 2, 371-376.

- Matamala, R. and Schlesinger, W. H. 2000. Effects of elevated atmospheric CO<sub>2</sub> on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, 6, 967-979.
- Medhurst, J., Parsby, J., Linder, S., Wallin, G., Ceschia, E. and Slaney, M. 2006. A whole-tree chamber system for examining tree-level physiological responses of field-grown trees to environmental variation and climate change. *Plant Cell and Environment*, 29, 1853-1869.
- Meigs, G. W., Donato, D. C., Campbell, J. L., Martin, J. G. and Law, B. E. 2009. Forest fire impacts on carbon uptake, storage, and emission: the role of burn severity in the Eastern Cascades, Oregon. *Ecosystems*, 12, 1246-1267.
- Melillo, J. M., Aber, J. D., Linkins, A. E., Ricca, A., Fry, B. and Nadelhoffer, K. J. 1989. Carbon and Nitrogen dynamics along the decay continuum: plant litter to soil organic matter. *Plant and Soil*, 115, 189-198.
- Melillo, J. M., Aber, J. D. and Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, 63, 621-626.
- Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., Catricala, C., Magill, A., Ahrens, T. and Morrisseau, S. 2002. Soil warming and carbon-cycle feedbacks to the climate system. *Science*, 298, 2173-2176.
- Mencuccini, M. and Hölttä, T., 2010. The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytologist*, 185, 189-203.
- Millard, P., Midwood, A. J., Hunt, J. E., Whitehead, D. and Boutton, T. W. 2008. Partitioning soil surface CO<sub>2</sub> efflux into autotrophic and heterotrophic components, using natural gradients in soil δ<sup>13</sup>C in an undisturbed savannah soil. *Soil Biology & Biochemistry*, 40, 1575-1582.
- Morales, P., Hickler, T., Rowell, D. P., Smith, B. and T Sykes, M. 2007. Changes in European ecosystem productivity and carbon balance driven by regional climate model output. *Global Change Biology*, 13, 108-122.
- Moyes, A. B., Schauer, A. J., Siegwolf, R. T. W. and Bowling, D. R. 2010. An injection method for measuring the carbon isotope content of soil carbon dioxide and soil respiration with a tunable diode laser absorption spectrometer. *Rapid Communications in Mass Spectrometry*, 24, 894-900.
- Muhr, J., Goldberg, S. D., Borken, W. and Gebauer, G. 2008. Repeated drying-rewetting cycles and their effects on the emission of CO<sub>2</sub>, N<sub>2</sub>O, NO, and CH<sub>4</sub> in a forest soil. *Journal of Plant Nutrition and Soil Science*, 171, 719-728.
- Nagy, J., Lewin, K. F., Hendrey, G. R., Hassinger, E. and Lamorte, R. 1994. FACE facility CO<sub>2</sub> concentration control and CO<sub>2</sub> use in 1990 and 1991. *Agricultural and Forest Meteorology*, 70, 31-48.
- Ngao, J., Bernhard-Reversat, F. and Loumeto, J. J. 2009. Changes in eucalypt litter quality during the first three months of field decomposition in a Congolese plantation. *Applied Soil Ecology*, 42, 191-199.
- Niinisto, S. M., Silvola, J. and Kellomaki, S. 2004. Soil CO<sub>2</sub> efflux in a boreal pine forest under atmospheric CO<sub>2</sub> enrichment and air warming. *Global Change Biology*, 10, 1363-1376.

- Niu, S. L. and Wan, S. Q. 2008. Warming changes plant competitive hierarchy in a temperate steppe in northern China. *Journal of Plant Ecology-Uk*, 1, 103-110.
- Norby, R., Warren, J., Iversen, C., Garten, C., Medlyn, B. and Mcmurtrie, R. 2009. CO<sub>2</sub> enhancement of forest productivity constrained by limited Nitrogen availability. *Nature Precedings* [Online]. [Accessed 24 Jun 2010].
- Norby, R. J. and Luo, Y. Q. 2004. Evaluating ecosystem responses to rising atmospheric CO<sub>2</sub> and global warming in a multi-factor world. *New Phytologist*, 162, 281-293.
- Norby, R. J., Wullschleger, S. D., Gunderson, C. A., Johnson, D. W. and Ceulemans, R. 1999. Tree responses to rising CO<sub>2</sub> in field experiments: implications for the future forest. *Plant Cell and Environment*, 22, 683-714.
- Norris, T. S., Bailey, B. J., Lees, M. and Young, P. 1996. Design of a controlled-ventilation open-top chamber for climate change research. *Journal of Agricultural Engineering Research*, 64, 279-288.
- Nottingham, A. T., Griffiths, H., Chamberlain, P. M., Stott, A. W. and Tanner, E. V. J. 2009. Soil priming by sugar and leaf-litter substrates: A link to microbial groups. *Applied Soil Ecology*, 42, 183-190.
- Oberbauer, S. F., Oechel, W. C. and Riechers, G. H. 1986. Soil respiration of Alaskan tundra at elevated atmospheric concentration. *Plant and Soil*, 96, 145-148.
- O'donnell, J. A., Turetsky, M. R., Harden, J. W., Manies, K. L., Pruett, L. E., Shetler, G. and Neff, J. C. 2009. Interactive effects of fire, soil climate, and moss on CO<sub>2</sub> fluxes in black spruce ecosystems of interior Alaska. *Ecosystems*, 12, 57-72.
- Ohlinger, R. 1995. Biomass-C by Fumigation-Extraction Technique. In: Schinner, F., Ohlinger, R., Kandeler, E. & Margesin, R. (eds.) *Methods in Soil Biology*. Berlin: Springer
- Olsson, P., Linder, S., Giesler, R. and Hogberg, P. 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. *Global Change Biology*, 11, 1745-1753.
- Orchard, V. A. and Cook, F. J. 1983. Relationship between soil respiration and soil moisture. *Soil Biology & Biochemistry*, 15, 447-453.
- Pajari, B. 1995. Soil respiration in a poor upland site of Scots pine stand subjected to elevated temperatures and atmospheric carbon concentration. *Plant and Soil*, 168-169, 563-570.
- Pataki, D. E., Ellsworth, D. S., Evans, R. D., Gonzalez-Meler, M., King, J., Leavitt, S. W., Lin, G. H., Matamala, R., Pendall, E., Siegwolf, R., Van Kessel, C. and Ehleringer, J. R. 2003. Tracing changes in ecosystem function under elevated carbon dioxide conditions. *Bioscience*, 53, 805-818.
- Paterson, E., Midwood, A. J. and Millard, P. 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. *New Phytologist*, 184, 19-33.
- Pendall, E., Del Grosso, S., King, J. Y., Lecain, D. R., Milchunas, D. G., Morgan, J. A., Mosier, A. R., Ojima, D. S., Parton, W. A., Tans, P. P. and White, J. W.

- C. 2003. Elevated atmospheric CO<sub>2</sub> effects and soil water feedbacks on soil respiration components in a Colorado grassland. *Global Biogeochemical Cycles*, 17, 13.
- Phillips, R. P. and Fahey, T. J. 2005. Patterns of rhizosphere carbon flux in sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) saplings. *Global Change Biology*, 11, 983-995.
- Plain, C., Gerant, D., Maillard, P., Dannoura, M., Dong, Y. W., Zeller, B., Priault, P., Parent, F. and Epron, D. 2009. Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tuneable diode laser absorption spectrometer after in situ <sup>13</sup>CO<sub>2</sub> pulse labelling of 20-year-old beech trees. *Tree Physiology*, 29, 1433-1445.
- Powers, H. H., Hunt, J. E., Hanson, D. T. and McDowell, N. G. 2010. A dynamic soil chamber system coupled with a tunable diode laser for online measurements of δ<sup>13</sup>C, δ<sup>18</sup>O, and efflux rate of soil-respired CO<sub>2</sub>. *Rapid Communications in Mass Spectrometry*, 24, 243-253.
- Pregitzer, K., Loya, W., Kubiske, M. and Zak, D. 2006. Soil respiration in northern forests exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia*, 148, 503-516.
- Prevost-Boure, N. C., Soudani, K., Damesin, C., Berveiller, D., Lata, J. C. and Dufrene, E. 2010. Increase in aboveground fresh litter quantity overstimulates soil respiration in a temperate deciduous forest. *Applied Soil Ecology*, 46, 26-34.
- Pritchard, S. G., Strand, M. a. E., McCormack, L., Davis, M. A., Finzi, A. C., Jackson, R. B., Matamala, R., Rogers, H. H. and Oren, R. a. M. 2008. Fine root dynamics in a loblolly pine forest are influenced by free-air-CO<sub>2</sub>-enrichment: a six-year-minirhizotron study. *Global Change Biology*, 14, 588-602.
- Raich, J. W., Ewel, J. J. and Olivera, M. 1995. Soil CO<sub>2</sub> efflux in simple and diverse ecosystems on a volcanic soil in Costa Rica. *Turrialba*, 35, 33-42.
- Raich, J. W. and Potter, C. S. 1995. Global patterns of carbon-dioxide emissions from soils. *Global Biogeochemical Cycles*, 9, 23-36.
- Raich, J. W., Potter, C. S. and Bhagawati, D. 2002. Interannual variability in global soil respiration, 1980-94. *Global Change Biology*, 8, 800-812.
- Raich, J. W. and Schlesinger, W. H. 1992. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus Series B-Chemical and Physical Meteorology*, 44, 81-99.
- Raich, J. W. and Tufekcioglu, A. 2000. Vegetation and soil respiration: Correlations and controls. *Biogeochemistry*, 48, 71-90.
- Raison, R. J. 1979. Modification of the soil environment by vegetation fires, with particular reference to Nitrogen transformations: a review. *Plant and Soil*, 51, 73-108.
- Richards, A. E., Dathe, J. and Cook, G. D. 2012. Fire interacts with season to influence soil respiration in tropical savannas. *Soil Biology and Biochemistry*, 53, 90-98.

- Rogers, H. H., Runion, G. B. and Krupa, S. V. 1994. Plant-responses to atmospheric CO<sub>2</sub> enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, 83, 155-189.
- Ruehr, N. K. and Buchmann, N. 2010. Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. *Tree Physiology*, 30, 165-176.
- Ruehr, N. K., Offermann, C. A., Gessler, A., Winkler, J. B., Ferrio, J. P., Buchmann, N. and Barnard, R. L. 2009. Drought effects on allocation of recent carbon: from beech leaves to soil CO<sub>2</sub> efflux. *New Phytologist*, 184, 950-961.
- Ruess, R. W., Cleve, K. V., Yarie, J. and Viereck, L. A. 1996. Contributions of fine root production and turnover to the carbon and nitrogen cycling in taiga forests of the Alaskan interior. *Canadian Journal of Forest Research*, 26, 1326-1336.
- Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J. and Gurevitch, J. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 126, 543-562.
- Rustad, L. E. 2008. The response of terrestrial ecosystems to global climate change: Towards an integrated approach. *Science of the Total Environment*, 404, 222-235.
- Rustad, L. E. and Fernandez, I. J. 1998. Experimental soil warming effects on CO<sub>2</sub> and CH<sub>4</sub> flux from a low elevation spruce-fir forest soil in Maine, USA. *Global Change Biology*, 4, 597-605.
- Rustad, L. E., Huntington, T. G. and Boone, R. D. 2000. Controls on soil respiration: Implications for climate change. *Biogeochemistry*, 48, 1-6.
- Saiz, G., Green, C., Butterbach-Bahl, K., Kiese, R., Avitabile, V. and Farrell, E. 2006. Seasonal and spatial variability of soil respiration in four Sitka spruce stands. *Plant and Soil*, 287, 161-176.
- Sayer, E. J., Heard, M. S., Grant, H. K., Marthews, T. R. and Tanner, E. V. J. 2011. Soil carbon release enhanced by increased tropical forest litterfall. *Nature Clim. Change*, 1, 304-307.
- Sayer, E. J., Powers, J. S. and Tanner, E. V. J. 2007. Increased litterfall in tropical forests boosts the transfer of soil CO<sub>2</sub> to the atmosphere. *Plos One*, 2, 6.
- Sayer, E. J. and Tanner, E. V. J. 2010. A new approach to trenching experiments for measuring root-rhizosphere respiration in a lowland tropical forest. *Soil Biology & Biochemistry*, 42, 347-352.
- Schaefer, D. A., Feng, W. T. and Zou, X. M. 2009. Plant carbon inputs and environmental factors strongly affect soil respiration in a subtropical forest of southwestern China. *Soil Biology & Biochemistry*, 41, 1000-1007.
- Schenk, H. J. and Jackson, R. B. 2002. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. *Journal of Ecology*, 90, 480-494.



- Schindlbacher, A., Zechmeister-Boltenstern, S. and Jandl, R. 2009. Carbon losses due to soil warming: do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology*, 15, 901-913.
- Schlesinger, W. H. and Andrews, J. A. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry*, 48, 7-20.
- Schlesinger, W. H. and Lichter, J. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO<sub>2</sub>. *Nature*, 411, 466-469.
- Schulze, E. D., Wirth, C. and Heimann, M. 2000. Climate change - managing forests after Kyoto. *Science*, 289, 2058-2059.
- Singh, B. P., Rémy De Courcelles, V. and Adams, M. A. 2011. Soil respiration in future global change scenarios. In: Singh, B. P., Cowie, A. L. and Chan, K. Y. (eds.) *Soil Health and Climate Change*. Springer Berlin Heidelberg.
- Smart, D. R. and Penuelas, J. 2005. Short-term CO<sub>2</sub> emissions from planted soil subject to elevated CO<sub>2</sub> and simulated precipitation. *Applied Soil Ecology*, 28, 247-257.
- Solomon, S., Qin, D., Manning, M., Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., Chidthaisong, A., Gregory, J. M., Hegerl, G. C., Heimann, M., Hewitson, B., Hoskins, B. J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Somerville, R., Stocker, T. F., Whetton, P., Wood, R. A. and Wratt, D. 2007. 2007: Technical Summary. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H. L. (eds.) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press.
- Sponseller, R. A. 2007. Precipitation pulses and soil CO<sub>2</sub> flux in a Sonoran Desert ecosystem. *Global Change Biology*, 13, 426-436.
- Stape, J. L., Binkley, D. and Ryan, M. G. 2008. Production and carbon allocation in a clonal Eucalyptus plantation with water and nutrient manipulations. *Forest Ecology and Management*, 255, 920-930.
- Stern, H., Campbell, B. and Pollock, J. 2011. Victoria in 2010: Fifth wettest year on record [Online]. Melbourne: Bureau of Meteorology, Available: <http://www.bom.gov.au/climate/current/annual/vic/summary.shtml#top> [Accessed 1 February 2011].
- Stocks, B. J., Fosberg, M. A., Lynham, T. J., Mearns, L., Wotton, B. M., Yang, Q., Jin, J. Z., Lawrence, K., Hartley, G. R., Mason, J. A. and Mckenney, D. W. 1998. Climate change and forest fire potential in Russian and Canadian boreal forests. *Climatic Change*, 38, 1-13.
- Subke, J. A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N. and Cotrufo, M. F. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia*, 139, 551-559.

- Subke, J. A., Inghima, I. and Cotrufo, M. F. 2006. Trends and methodological impacts in soil CO<sub>2</sub> efflux partitioning: A metaanalytical review. *Global Change Biology*, 12, 921-943.
- Subke, J. A., Vallack, H. W., Magnusson, T., Keel, S. G., Metcalfe, D. B., Hogberg, P. and Ineson, P. 2009. Short-term dynamics of abiotic and biotic soil <sup>13</sup>CO<sub>2</sub> effluxes after in situ <sup>13</sup>CO<sub>2</sub> pulse labelling of a boreal pine forest. *New Phytologist*, 183, 349-357.
- Sulzman, E. W., Brant, J. B., Bowden, R. D. and Lajtha, K. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO<sub>2</sub> efflux in an old growth coniferous forest. *Biogeochemistry*, 73, 231-256.
- Swift, M. J., Heal, O. W. and Anderson, J. M. 1979. *Decomposition in Terrestrial Ecosystems*, Oxford, Blackwell Scientific Publications.
- Tang, X. L., Zhou, G. Y., Liu, S. G., Zhang, D. Q., Liu, S. Z., Li, J. and Zhou, C. Y. 2006. Dependence of soil respiration on soil temperature and soil moisture in successional forests in southern China. *Journal of Integrative Plant Biology*, 48, 654-663.
- Thornton, B., Paterson, E., Midwood, A. J., Sim, A. and Pratt, S. M. 2004. Contribution of current carbon assimilation in supplying root exudates of *Lolium perenne* measured using steady-state <sup>13</sup>C labelling. *Physiologia Plantarum*, 120, 434-441.
- Tingey, D. T., Lee, E. H., Waschmann, R., Johnson, M. G. and Rygielwicz, P. T. 2006. Does soil CO<sub>2</sub> efflux acclimatize to elevated temperature and CO<sub>2</sub> during long-term treatment of Douglas-fir seedlings? *New Phytologist*, 170, 107-118.
- Tingey, D. T., Phillips, D. L. and Johnson, M. G. 2000. Elevated CO<sub>2</sub> and conifer roots: effects on growth, life span and turnover. *New Phytologist*, 147, 87-103.
- Trueman, R. J. and Gonzalez-Meler, M. A. 2005. Accelerated belowground C cycling in a managed agriforest ecosystem exposed to elevated carbon dioxide concentrations. *Global Change Biology*, 11, 1258-1271.
- Vance, E. D., Brookes, P. C. and Jenkinson, D. S. 1987. An extraction method for measuring soil microbial biomass-C. *Soil Biology & Biochemistry*, 19, 703-707.
- Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldberg, O. and Bairlein, F. 2002. Ecological responses to recent climate change. *Nature*, 416, 389-395.
- Wan, S., Norby, R. J., Ledford, J. and Weltzin, J. F. 2007. Responses of soil respiration to elevated CO<sub>2</sub>, air warming, and changing soil water availability in a model old-field grassland. *Global Change Biology*, 13, 2411-2424.
- Wan, S. Q., Hui, D. F., Wallace, L. and Luo, Y. Q. 2005. Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie. *Global Biogeochemical Cycles*, 19, 13.

- Wan, S. Q. and Luo, Y. Q. 2003. Substrate regulation of soil respiration in a tallgrass prairie: Results of a clipping and shading experiment. *Global Biogeochemical Cycles*, 17, 12.
- Warren, J. M., Iversen, C. M., Garten, C. T., Norby, R. J., Childs, J., Brice, D., Evans, R. M., Gu, L., Thornton, P. and Weston, D. J. 2012. Timing and magnitude of C partitioning through a young loblolly pine (*Pinus taeda* L.) stand using <sup>13</sup>C labeling and shade treatments. *Tree Physiology*, 32, 799-813.
- Williams, A. a. J., Karoly, D. J. and Tapper, N. 2001. The sensitivity of Australian fire danger to climate change. *Climatic Change*, 49, 171-191.
- Williams, J. and Woinarski, J. 1997. *Eucalypt ecology: individuals to ecosystems*, Cambridge, Cambridge University Press.
- Woods, P. V. and Raison, R. J. 1983. Decomposition of litter in subalpine forests of *Eucalyptus delegatensis*, *Eucalyptus pauciflora* and *Eucalyptus dives*. *Australian Journal of Ecology*, 8, 287-299.
- Wu, J., Brookes, P. C. and Jenkinson, D. S. 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. *Soil Biology & Biochemistry*, 25, 1435-1441.
- Yuste, J. C., Janssens, I. A., Carrara, A., Meiresonne, L. and Ceulemans, R. 2003. Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest. *Tree Physiology*, 23, 1263-1270.
- Zak, D. R., Pregitzer, K. S., King, J. S. and Holmes, W. E. 2000. Elevated atmospheric CO<sub>2</sub>, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, 147, 201-222.
- Zhang, J., Griffis, T. J. and Baker, J. M. 2006. Using continuous stable isotope measurements to partition net ecosystem CO<sub>2</sub> exchange. *Plant Cell and Environment*, 29, 483-496.
- Zhang, W., Parker, K. M., Luo, Y., Wan, S., Wallace, L. L. and Hu, S. 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology*, 11, 266-277.
- Zhou, X. H., Sherry, R. A., An, Y., Wallace, L. L. and Luo, Y. Q. 2006. Main and interactive effects of warming, clipping, and doubled precipitation on soil CO<sub>2</sub> efflux in a grassland ecosystem. *Global Biogeochemical Cycles*, 20, 12.
- Zhu, B. A. and Cheng, W. X. 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology*, 17, 2172-2183.
- Zimmermann, M. and Bird, M. I. 2012. Temperature sensitivity of tropical forest soil respiration increase along an altitudinal gradient with ongoing decomposition. *Geoderma*, 187-188, 8-15.