

Waterlogging in the temperate
plantation species
Eucalyptus globulus and *E. nitens*

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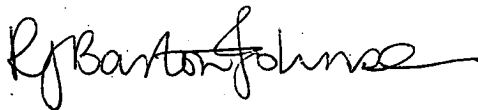
Submitted in fulfillment for the degree of Doctor of Philosophy

School of Plant Science
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~ Declaration ~

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of materials previously published or written by any other person, except where due reference is made in the text.

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Rebecca J Barton-Johnson

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Hobart

December 2006

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with the *Copyright Act 1968*.**

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~ CONTENTS ~

Abstract	iv
 Chapter 1	
General Introduction.....	1
 Chapter 2	
Response of <i>Eucalyptus globulus</i> to waterlogging under field Conditions	12
 Chapter 3	
The effect of long term waterlogging on the growth and physiology of <i>E. globulus</i> and <i>E. nitens</i>	58
 Chapter 4	
The effect of fertiliser mode and timing of application in alleviating waterlogging damage	109
 Chapter 5	
Hypoxic pre-conditioning of <i>E. globulus</i> and <i>E. nitens</i> seedlings prior to exposure to waterlogging	141
 Chapter 6	
Response of <i>E. globulus</i> and <i>E. nitens</i> to hypoxia under hydroponic conditions and the development of root porosity	166
 Chapter 7	
General Discussion.....	182
 Appendix	190
 References cited	195

~ ABSTRACT ~

This project investigated the effect of waterlogging on the productivity of *Eucalyptus globulus* under field conditions, and the relative tolerance of the two temperate plantation eucalypt species, *E. globulus* and *E. nitens*, from the subgenus *Symphyomyrtus*, to waterlogging.

In eucalypt plantations across Tasmania, waterlogging is a serious threat to productivity. It is estimated that at least 11 % of all commercial eucalypt plantations in Tasmania are affected to some degree, by waterlogging. The losses incurred by waterlogging during the first 2 years of plantation establishment were found to have significant long-term effects on tree productivity at two sites across the state.

In the field, soil indicators of long-term waterlogging, specifically the evidence of greying, in combination with current seasonal waterlogging and development of hypoxic soil conditions were found to be the factors closely associated with severe reductions in tree height and growth. The use of extensive soil mapping and assessment prior to plantation establishment are therefore effective tools in identifying potential waterlogging problems.

Under long-term waterlogging, there was no indication of the development of plant water stress during exposure to waterlogging. Waterlogged seedlings of both species exhibited reduced foliar nutrient status, with significantly reduced foliar nitrogen, phosphorus and potassium concentrations. Therefore, waterlogging-induced nutrient deficiency is a mechanism involved in the growth reduction of these species under waterlogged conditions.

Various methods of fertiliser application were investigated to determine the most effective mode in ameliorating waterlogging damage. Soil-based applications of slow release fertilisers applied either prior to, or after, a waterlogging event, were the most successful. Fertiliser application was associated with improved growth, foliar nutrition and increased aerenchymatous root production.

Reduced availability of nutrients in waterlogged soils and/or reduced uptake of these nutrients are the two possible mechanisms leading to the development of foliar nutrient deficiency of waterlogged plants. It was found that under hypoxic hydroponic culture, seedlings with low nutrient status were capable of an increased uptake of nutrients when transferred to high nutrient conditions. This result is significant as it identifies reduced availability of soil nutrients as the primary mechanism responsible for the development of foliar nutrient deficiency in *E. globulus* and *E. nitens* under waterlogged conditions.

The relationship between aerenchymatous root development and waterlogging tolerance was investigated. It was determined that singular measurements of root adaptive capacity should not be used as the sole measure of tolerance to waterlogging in these species. The regulation of growth and maintenance of relatively high photosynthetic rates are also useful indicators of tolerance. Using this array of measures, it was determined that *E. nitens* was inherently more waterlogging tolerant than *E. globulus*. *Eucalyptus globulus* seedlings demonstrated a greater potential for increased waterlogging tolerance by the application of fertiliser regimes.

~ CHAPTER 1 ~

General Introduction

1.1 Relevance of waterlogging to forestry industry

Waterlogging is defined as the total saturation of the soil matrix, without free water above the soil surface. The development and severity of waterlogging depends on a number of environmental factors including topography, soil type and structure, vegetation type and climate (Wigmosta et al. 1994). Site topography affects the movement and distribution of water through the landscape, with water accumulating in depressions, resulting in waterlogging (Barling et al. 1994). Soil properties including texture, structure and spatial heterogeneity affect the permeability of the soil to water, infiltration rates and water storage capacity (McLaren and Cameron 1994). Vegetation structure affects processes including stemflow, infiltration rate, hydraulic conductivity of soils (Baird and Wilby 1999) and evapotranspiration, which all affect the movement of water and therefore development of waterlogging. Rainfall is an important climatic variable, which in conjunction with infiltration, run-off and throughflow controls water input into a landscape. The development of waterlogging is both spatially and temporally (with strong seasonal effects) heterogeneous.

Waterlogging is a widespread and significant problem across much of Australia's arable land. It is potentially very damaging, reducing crop production and financial returns from agriculture. In Victoria, 3.8 million hectares of duplex soils used for cereal production are affected by waterlogging (Gibbs and Greenway 2003). It was estimated that there was a \$13 million loss in the value of the 1987 West Australian cereal crop that was attributable to waterlogging or flooding (McFarlane and Wheaton 1990). Pang et al. (2004) reported an estimated average annual \$180 million loss to the Australian economy due to waterlogging.

The recent expansion of hardwood forestry into land previously under traditional agricultural systems (annual cropping, pasture, grazing etc) has meant that the

impacts of waterlogging need to be assessed by forestry operators. The importance of using good site selection criteria, including the incidence and effect of waterlogging on timber production, in selecting potential sites for plantation establishment, cannot be overlooked. The incorporation of factors affecting the development of waterlogging into process-based models, used in the assessment of potential plantation sites, has now been achieved (Mummery and Battaglia 2001).

Tasmania has over 146,000 ha of hardwood plantations, 22% of the nation's total area (National Forest Inventory 2004). The total plantation estate has increased substantially over recent years, even though nationwide the area of new plantings has decreased since 2000. Since 1999, Tasmania has lead Australia with the largest area of newly established plantations (in 2003 this was approximately 9500 ha). The availability of suitable sites, at an affordable price, is a critical factor in the rate of plantation establishment, and is a significant constraint across south-west Victoria and south-east South Australia. This issue is certain to become a major constraint in all southern states, including Tasmania.

Eucalypt species that typically occupy habitats prone to waterlogging are from the *Symphyomyrtus* subgenus (Noble 1989). *Symphyomyrtus* species are more waterlogging tolerant than the species from the subgenera *Monocalyptus* (Davidson 1985, Noble 1989). The waterlogging tolerant *Symphyomyrtus* species include *E. camaldulensis*, *E. globulus* (Sena-Gomes and Kozlowski 1980), *E. ovata*, *E. gunnii*, *E. urnigera* and *E. johnstonii* (Davidson 1985). Waterlogging is a particular problem across of Victoria, South Australia and in Tasmania, where the major hardwood plantation species are *Eucalyptus globulus* and *E. nitens* (both from the *Symphyomyrtus* subgenus). These two species are selected for their superior early growth rate (Turnbull et al. 1993), but not for waterlogging resistance.

In Tasmania, sites with an annual rainfall of above 1000 mm year⁻¹ and altitude of 300-600 m are favoured for plantation establishment (Honeysett et al. 1996). At these high rainfall areas where drainage is poor there is potential for plantations to

be affected by waterlogging. Therefore there is a need to accurately quantify the response of *E. globulus* and *E. nitens* to waterlogging. Marcar et al. (2002) classified *E. nitens* as a more waterlogging tolerant species than *E. globulus*. The opposite was reported by Connell (1998) where the waterlogging tolerance of *E. globulus* was rated as the highest of the three species; *E. globulus* > *E. nitens* > *E. regnans*. Previous work has reported a severe reduction in the productivity of both *E. globulus* and *E. nitens* in waterlogged plantations. Mummery et al. (1999) reported a 55% reduction in the growth of an *E. nitens* plantation as a result of waterlogging. A 70% reduction in shoot dry weight of *E. globulus* in response to waterlogging was reported by Marcar et al. (2002).

Waterlogging can lead to a number of changes in site characteristics which add to the impact on the plantation productivity. These include soil compaction, reduced trafficability and increased weed infestation (resulting from poor weed control). Cold-air drainage is a major confounding factor in the identification and quantification of damage caused by waterlogging (Davidson and Reid 1987). It can be difficult to isolate these as individual factors affecting low-lying areas.

In hardwood plantations there is also the problem of the ephemeral development of waterlogging, which may only be present when the plantation is young. During the early years of plantation growth there is low transpirational capacity due to low stand biomass. As the plantation ages water demand increases and waterlogging becomes less of a problem. Often there are only relatively small and/or few pockets of waterlogging within a plantation, which can make the management of these areas difficult for plantation foresters.

Currently, the management practice for the establishment of plantations on waterlogged sites is strict avoidance. In the assessment of potential plantation sites, Forestry Tasmania classify the drainage class of soils and use the following descending scale; 1. Rapidly drained; 2. Well-drained; 3. Moderately well-drained; 4. Imperfectly drained; 5. Poorly drained and 6. Very poorly drained (Laffan 2002). In areas classified as having poor to very poor drainage status, plantation establishment is not recommended. In Tasmania, 11% of over 11,000

sites surveyed by Forestry Tasmania were classified as either imperfectly or poorly drained (Sam Rees, pers. comm.). This represents a substantial area of the plantation forestry estate.

1.2 Physiological effect of waterlogging

The development of waterlogging involves a series of inter-related factors, the most significant being the depletion of oxygen concentration in the soil. Oxygen deficiency is widely assumed to be the primary cause of waterlogging damage (Trought and Drew 1980; Marschner 1995). When water fills all the soil pores, the rate of diffusion of oxygen through the soil is reduced 10,000-fold, compared with gaseous diffusion. Following depletion of soil oxygen there are a number of changes that occur in both the soil and plant processes that affect the loss in productivity observed under waterlogged conditions. Saturation of the soil induces a number of chemical, physical and biological changes within the soil.

Oxygen deficit in the soil results in a switch from aerobic to anaerobic respiration. Consequently, there is a severe reduction in the maximum net yield of adenosine triphosphate (ATP). Aerobic respiration yields 36 moles of ATP per mole hexose whereas anaerobic respiration yields only 2 moles of ATP for each mole of hexose. Where metabolic demands remain unchanged during waterlogging, anaerobic respiration leads to a greater demand on respiratory substrates, and reserves are quickly depleted. This depletion of respiratory substrates leads to the development of an energy deficit in roots (Drew 1992; Gibbs and Greenway 2003), which limits active processes including the uptake and transport of essential plant nutrients.

In response to this switch to anaerobic metabolism, there is an accumulation of toxic end products. The accumulation of ethanol (major end product of anaerobic respiration) and other toxic chemicals arising from anaerobic respiration or microbial activity may lead to waterlogging injury (Drew and Sisworo 1979; Kozlowski 1979; Drew and Lynch 1980; Kozlowski 1984).

As a direct consequence of the decrease in dissolved oxygen, waterlogging results in a decrease in soil redox potential. Soil micro-organisms are forced to utilise other electron acceptors than oxygen, to facilitate respiratory oxidation (Armstrong 1975, Drew 1983). This results in the conversion of many elements and compounds into a chemically reduced state, and a subsequent decrease in soil redox potential. There is a sequential reduction of chemical species in the order; $O_2 > NO_3^- > Mn^{3+} > Fe^{3+} > SO_4^{2-} > CO_2$ (Morales et al. 2002, Patrick and Jugsujinda 1992).

Anaerobic conditions cause changes in the soil pH. Hydrogen ions are removed from solution due to anaerobic respiration, which in acid soils can cause the pH to rise. In anaerobic alkaline soils, the buildup of CO_2 from respiration is the dominant effect and causes the soil pH to drop. Most waterlogged soils eventually develop a near neutral pH of between 6.7 and 7.2 (McLaren and Cameron 1996).

Waterlogging reduces the concentration of PO_4^{3-} and NO_3^- in all soil types (Phillips and Greenway 1998). Nitrogen availability is lowest in acid soils due to the inhibition of nitrification as a result of the low activity of soil bacteria (McLaren and Cameron 1996). The form of the phosphate ion and rate of reaction of phosphate with other soil components also changes in response to changes in pH. Under acid conditions, the presence of high concentrations of soluble iron (Fe^{2+}), manganese (Mn^{2+}) and aluminium (Al^{3+}) results in the precipitation of insoluble phosphate compounds, and loss of available phosphorus (McLaren and Cameron 1996).

An increase in the soil pH affects the concentration and availability of micronutrients and other nutrients including calcium and potassium. The concentrations of iron, manganese, ammonia, calcium, magnesium, potassium and sodium are generally increased (Phillips and Greenway 1998). All of these changes to nutrient availability are dependent on the cation-exchange capacity (CEC) and buffering capacity of the soil. In addition to changes in nutrient availability, the accumulation of reduced substrates such as nitrites, organic acids,

sulphides, and divalent iron and manganese under anaerobic conditions are all injurious to plants *in vivo* (Kozłowski 1979).

Physiological consequences of waterlogging include reduced root permeability and water absorption and mineral uptake and transport (Grichko and Glick 2001). Typical morphological symptoms in response to waterlogging include retardation of plant growth, changes to biomass partitioning patterns, reduced root growth, leaf loss, adventitious root development and aerenchyma production.

The rapid response of plants to waterlogging indicates the involvement of plant hormones. Plant hormone synthesis and concentration are affected by waterlogging. There is an inhibition of gibberellin and cytokinin production (Jackson 2002), whilst ethylene biosynthesis is increased. The immediate precursor to ethylene, 1- aminocyclopropane - 1 - carboxylic acid (ACC) is transported from the roots to the shoots in the xylem of flooded plants. This results in the up-regulation of ethylene production and concentration in leaves and the development of leaf epinasty (Bradford and Yang 1981). Accelerated ethylene production is a common response of plants to environmental stresses, including drought, high temperature and insect attack (Tang and Kozłowski 1984).

The accumulation of ethylene in plant tissues is associated with many morphological symptoms of, and adaptations to waterlogging; leaf senescence and abscission, inhibited leaf initiation and expansion, leaf chlorosis, reduced stem height, formation of callus, reduced root growth, suppression of secondary growth, adventitious roots (originating from the stem) and aerenchyma development (the production of large cortical air spaces that facilitate the diffusion of oxygen and other gases through the plant body) in both the stem and root systems (Bradford and Yang 1981; Kawase 1981; Kozłowski 1979, 1984; Bacanamwo and Purcell 1999; Armstrong et al. 1991; Voesenek et al. 1992). Abscissic acid (ABA) also has a role in the waterlogging response, with waterlogged plants having a higher concentration of ABA (Bradford and Yang

1981). The application of exogenous ABA may result in an increased tolerance to hypoxia (Kato-Noguchi 2000).

Physiological responses to waterlogging generally occur very rapidly. Stomatal closure occurs quickly (Sun et al. 1995) with a rapid decline in photosynthetic rate (Kozlowski 1984). This decrease is attributed to a decrease in leaf water potential and an increase in stomatal resistance, and a number of other processes such as changes in hormone concentrations and the transport of photosynthates (Bishnoi and Krishnamoorthy 1992). These processes are species specific, with tolerant species re-opening stomata after a short period of time (hours), which results in continued growth throughout waterlogged conditions (Pezeshki et al. 1996).

Early studies reported a decrease in leaf water potential (Ψ), which was assumed to be responsible for early stomatal closure and the reduction in photosynthesis. In many species, there is no evidence of low water potential as a result of waterlogging (Zang and Davies 1986). Stomatal closure, a typical response of waterlogged plants is not necessarily induced by reduced hydraulic conductivity, but may be triggered by a hormonal signal from the roots (Nicolas et al. 2005). This appears to be the case in *Eucalyptus* (Pereira and Kozlowski 1977, Close and Davidson 2003).

Many studies have described a rapid decrease in the foliar chlorophyll content of waterlogged plants (Pang et al. 2004, Bishnoi and Krishnamoorthy 1992, Ashraf and Yasmin 1991, Pasrija and Chithara 1988). This rapid decrease in chlorophyll content may be indicative of the development of photoinhibition. Photoinhibition is defined as the light-dependent inhibition of the light reactions of photosynthesis (Long et al. 1994). If excess light is absorbed (i.e. excess to the amount that the plant is capable of utilizing or dissipating) the excess energy will result in oxidative damage to Photosystem II of photosynthesis (Foyer et al. 1994). It has been shown to develop in plants under a range of stresses including low temperatures, excessive light, nutrient deficiency and drought with a sustained decrease in the efficiency of Photosystem II and reduced photosynthetic capacity (Berry and Bjorkman 1980). Photoinhibition has been shown to develop in

waterlogged *E. nitens* seedlings (Close and Davidson 2003). Photoinhibited plants show a decrease in quantum yield of photosynthesis and this can be measured as a decrease in the variable to maximal chlorophyll fluorescence ratio (F_v/F_m) (Close et al. 2000).

1.3 Morphological adaptations to waterlogging

The most significant morphological adaptations are the development of adventitious and aerenchymatous roots. Aerenchyma is the development of air spaces within the cortex of the stem (hypertrophy) and root, which acts as an internal air-conduction system (Kawase and Whitmoyer 1980). It can develop by two processes; (i) schizogenous aerenchyma results from cell separation, through differential development; and (ii) lysigenous aerenchyma is formed through cell death (Evans 2003). Aerenchyma is produced in response to a number of environmental stresses other than waterlogging, including high temperatures, drought, nutrient deficiency and hypoxia (Evans 2003, Fan et al. 2003).

Aerenchyma confers two significant advantages to waterlogged root systems. It provides a means for transporting oxygen from the atmosphere to the roots. It also works to alleviate respiratory demand by reducing cell volume (Evans 2003). The effectiveness of aerenchyma can be increased through the formation of epidermal barriers impermeable to gases, to prevent the leakage of oxygen from the roots (Visser et al. 2003).

The formation of hypertrophied lenticels may be an important morphological adaptation to waterlogging in some plant species. Lenticels are pores in the stem which can assist in the aeration of stem tissue, and may provide an outlet for the removal of toxic gases (Hook et al. 1983; Tang and Kozlowski 1984). Other mechanisms of tolerance include the induction of a number of anaerobic proteins (ANPs), which enable energy-generating metabolism to proceed in the absence of oxygen (Visser et al. 2003). Numerous proteins have been identified as being associated with the waterlogging response, in both tolerant and in-tolerant species. The majority of these proteins are enzymes involved in glycolysis and

fermentation or in carbohydrate mobilization and catabolism (Vartapetian et al. 2003, Vartapetian and Jackson 1997).

The response of a species to waterlogging may be affected by plant age, water chemistry, time and duration of exposure, hypoxic pre-treatment, carbohydrate status and temperature (Nicolas et al. 2005, Gibbs and Greenway 2003). The continued survival and maintenance of growth are the two broad factors in defining tolerance to waterlogging. Maintenance of growth under waterlogged conditions occurs only in tolerant species, and then usually only in the shoot (Gibbs and Greenway 2003). Therefore a better criterion for the assessment of tolerance is the ability to survive exposure to hypoxic/anoxic conditions, and the ability to recover physiologically and metabolically upon re-aeration (Nicolas et al. 2005, Gibbs and Greenway 2003). Tolerant species have the ability to rapidly resume growth once root aeration is restored (Crawford 1982). The degree of development of adaptive mechanisms to waterlogging is a major factor affecting the level of survival, growth and recovery from waterlogging.

1.4 Mechanisms of waterlogging damage

A number of possible mechanisms have been proposed for the development of waterlogging injury. These include the accumulation of toxic metabolic by-products (Crawford 1978), cytoplasmic acidosis (Davies and Kozlowski 1974), energy deficit, post-anoxic injury from free radical accumulation, inhibition of the transport of water, minerals and hormones from roots to shoots and the development of water stress (Kramer and Jackson 1954, Jackson et al. 2003).

As a consequence of the inhibition of water and mineral transport, nutrient deficiency may develop under waterlogged conditions. Nutrient deficiency results in a reduced leaf area and number, and a reduction in the concentration of foliar chlorophyll, which is also a common response to waterlogging (Pasrija and Chithara 1988; Ashraf and Yasmin 1991; Bishnoi and Krishnamoorthy 1992, Pang et al. 2004). This rapid reduction in chlorophyll content after the onset of waterlogging may result in a decreased photosynthetic rate.

Waterlogging-induced nutrient deficiency may be responsible for the reductions in tree growth, development of physiological stress and photoinhibition. Nutrient deficiency has been reported in a number of species under waterlogged conditions, including soybean (Bacanamwo and Purcell 1999), loblolly pine (Hook et al. 1983), maize (Lizaso et al. 2001), wheat (Malik et al. 2001) and *Eucalyptus nitens* (Close and Davidson 2003). The waterlogging tolerance of a particular species also affects its nutritional response. Less waterlogging-tolerant species exhibit reduced nitrogen uptake and metabolism under waterlogged conditions compared with more tolerant species (Kreuzwieser et al. 2002).

Waterlogging affects both the availability of nutrients and the capacity of root systems to absorb and transport water and mineral nutrients (Morard and Silvestre 1996; Lizaso et al. 2001). Nutrient uptake by plant roots is a function of root absorption capacity and the mobility/availability of ions within the soil (Grove et al. 1996). As discussed, waterlogging induces a number of chemical changes in soils, resulting in changes to the state and availability of essential plant nutrients. It also has a severe effect on plant root function. Intolerant species suffer extensive root death as a result of soil anoxia, which limits soil exploration and the acquisition of nutrients. Waterlogging also affects microbial activity, in particular, the association of mycorrhizae with plant roots. This will specifically affect the nitrogen and phosphorus uptake of eucalypt roots, which are reliant upon these associations (Grove et al. 1996).

Therefore alterations in soil and plant nutrition are critical components of waterlogging damage, and present a potential method of amelioration of waterlogging damage through application of specific fertilisation regimes. Application of nitrogen fertilisers has been reported to reduce waterlogging damage and increase the rate of recovery in barley (Drew et al. 1979), maize (Chen 1989), sorghum (Singh et al. 2002) and rice (Carreres et al. 2003). Foliar application of fertilisers has also been examined by Zhou et al. (1997) and reported to reduce the severity of waterlogging damage in winter rape.

1.5 Aims of this study;

1. To quantify and compare the tolerance of *E. globulus* and *E. nitens* to waterlogging in terms of growth, physiology and adaptive mechanisms.
2. To quantify the loss in productivity of *E. globulus* seedlings in waterlogged plantations and suggest mechanisms to ameliorate this.
3. Investigate the role of photoinhibition under waterlogged conditions.
4. Assess the relationship between waterlogging, changes in soil and plant nutrition and eucalypt productivity.
5. Assess the potential for amelioration of waterlogging damage through fertilisation regimes and investigate the optimal mode and timing of application to alleviate waterlogging stress.

~ CHAPTER 2 ~

Response of *Eucalyptus globulus* to waterlogging in the field**2.1 Introduction**

Tasmania has a total 146640 ha of hardwood plantations, which is over 21% of the Australia's plantation estate (National Plantation Inventory Update 2004). The state also has the largest area of newly established hardwood plantations, with a total of 9500 ha converted to plantation hardwood forestry during 2003 (National Plantation Inventory Update 2004). Considering the size of the forestry industry within Tasmania, particularly the hardwood plantation estate, any threat to the overall productivity and economic return represents a major financial loss to the state. Mummery et al. (2001) demonstrated that even minor changes in annual plantation productivity will have a major effect on the rate of return at harvest.

The availability of suitable land at an affordable price, for plantation establishment has become the major limiting factor for the forestry industry, particularly in the green triangle of south-western Victoria and in south-eastern South Australia, with the problem becoming more apparent within the Tasmanian forestry industry. Many potential plantation sites have been classified as poorly drained, significantly restricting the area available for plantation establishment. In the Pipers region, in north-eastern Tasmania, 25 % of the area deemed unsuitable for plantation establishment was classified as having soils with poor drainage (Laffan et al. 1994).

Identification of waterlogging can be difficult due to its ephemeral nature. It is also often localised in small areas and easily overlooked during site assessment. For obvious reasons, plantation managers tend to select sites with high rainfall and flat topography for plantation establishment. However, these two factors are often associated with the development of waterlogging, particularly during early plantation growth when transpirational demand is low. Therefore, the

productivity of large areas of plantations may be compromised by previously unidentified areas of localised ephemeral waterlogging during the early years of establishment.

Forest managers gain evidence of waterlogging, which may limit plantation productivity, through soil inspections conducted during site assessment. Soil gleying is the process of chemical reduction within the soil profile which occurs under anaerobic conditions. The reduction of ferric iron oxides (yellow colour) to the more soluble ferrous ion (grey colour) results in the soil becoming dominated by grey/green colours. The process of gleying is typically identified by “greying” of the soil profile. The degree of greying (the percentage of soil volume with grey colouring) is indicative of the drainage status of the soil and evidence of long-term saturation of the soil (Van den Berg and Loch 2000). Soils with > 50% greying of the soil profile to a depth of 60 cm are classified as poorly-drained; and soils with highly organic topsoils overlaying predominantly grey subsoils are classified as very poorly-drained (Laffan 2002). Sites identified as having one of these drainage classes are classified as low productivity sites, and therefore not suitable for plantation establishment (Laffan 2002).

The seasonality of waterlogging, and the timing and duration of its development are important factors influencing the survival, growth and biomass partitioning of affected plants (Kaelke and Dawson 2003). In temperate zones of the southern hemisphere, waterlogging typically develops in early winter (June/July) as a consequence of high rainfall and low evaporation. High water tables may be present until mid-spring (October/November), corresponding to the period of maximum growth rate in plantation trees. Waterlogging results in root pruning and loss of leaf biomass (Kozlowski 1979), and therefore the effects of waterlogging on tree growth and productivity may persist for longer than the waterlogging event. It is necessary to assess the growth and physiology of waterlogged trees throughout the year in order to adequately characterise the short and long-term effects of waterlogging on tree physiology and productivity.

It has been demonstrated that eucalypt species from the sub-genus *Symphyomyrtus* (e.g. *E. johnstonii*, *E. gunnii*, *E. urnigera*) are more tolerant of waterlogging than the species from the sub-genus *Monocalyptus* (eg. *E. pulchella*, *E. coccifera*, *E. delegatensis*) (Davidson 1985). This response is primarily attributed to the greater capacity to develop adventitious and aerenchymatous roots of *Symphyomyrtus* species (Davidson 1985, Van der Moezel et al. 1988).

The typical morphological responses of eucalypt species to waterlogging include the development of adventitious roots, stem hypertrophy and production of aerenchymatous roots (Sena Gomes and Kozłowski 1980) and leaf loss (Ladiges and Kelso 1977). Van der Moezel et al. (1988) reported a reduction in seedling height of up to 80% in five eucalypt species (*E. comitea-valis*, *E. kondininensis*, *E. lesouefii*, *E. platycorys* and *E. spathulata*). The waterlogging tolerance of other species which have been investigated includes *E. ovata* and *E. viminalis* (Ladiges and Kelso 1977), *E. robusta* (Clemens and Pearson 1977) and *E. grandis* and *E. saligna* (Clemens et al. 1978). Despite this, there is limited knowledge of the effect of waterlogging on production forestry, particularly on the growth of temperate eucalypt species in the field. *Eucalyptus nitens* has been reported to show a 50% reduction in tree height (Connell 1998) and 55% reduction in biomass (Mummery et al. 1999) under waterlogged conditions in the field.

The aim of this chapter is to quantify the effect of waterlogging on the growth and productivity of *E. globulus* in commercial plantations.

Hypotheses tested;

1. Reduced height and biomass of *E. globulus* trees in plantation is associated with the annual cycle of waterlogging as indicated by soil structure and colour, elevated soil water level and depressed pH, dissolved oxygen and redox potential.
2. Reduced tree growth and productivity is associated with reduced photosynthetic rates, stomatal conductance, leaf water potential and predawn chlorophyll fluorescence.
3. Waterlogging has a seasonal effect on tree productivity.
4. Management techniques can be developed to reduce the effect of waterlogging on eucalypt plantations.

2.2 Materials and methods

2.2.1 Site A

Site selection

In May 2002, “Loloma” a plantation near at Nabowla in northeast Tasmania was selected as the primary field site (map reference: map sheet Nabowla 5244 1:25000). Loloma was a 480 ha site at 50 m.a.s.l that had previously been managed as farmland, comprised of a mosaic of improved pasture for grazing, cropping and native forest. The site was prepared for plantation using a broadcast spray of knockdown herbicide (June 2001), cultivation and mounding, rotary hoeing and application of a residual herbicide before planting with *Eucalyptus globulus* in August 2001.

A detailed soil and geological survey was conducted by Forestry Tasmania in 1999. This reported the underlying rock to be Silurian-Devonian sandstones/siltstones (Mathinna beds). The areas selected for study were patches of poorly growing plantation, which in November 2000, had free surface water present. Despite these isolated pockets of waterlogging, the majority of the plantation area was deemed suitable for plantation establishment. These patches were on sandy loam soils overlaying clay, categorised as a Baker profile (Laffan 1999). This soil type is characterised by organic-rich topsoils overlaying grey clay subsoils and are typically nutrient poor, though fertilisation during farming had increased the nutrient status locally. Baker soils are poorly drained and occur in wet depressions formed on alluvium derived from Mathinna Bed sedimentary rock (Laffan et al. 1995).

Mean annual rainfall obtained from the nearest weather station (within 1 Km) was 1003 mm.

Plot description

Three study areas, all within 500 m, were identified at Loloma. Study areas were positioned on a flat river terrace beside the Little Forester River. At each study area a waterlogged and control plot, each of 16 trees, was pegged out. At study

area 1, a 100 m transect was also run west to east from the waterlogged region, where trees were stunted, to the healthy and productive trees only 100 m from the river edge. Six plots (Tr1 to Tr6) were established along this transect at 20 m intervals, representing a gradient in tree performance, and associated with changes in soil water status, from waterlogged at Tr1 to drained at Tr6. Study areas 2 and 3 were located approximately 200 and 300 m north, respectively. The control plots for areas 2 and 3 were within 30 m of the waterlogged plots for these areas. A photograph of study area 1 is presented as Plate 2.1. Waterlogged plots had free surface water in winter and poor tree productivity (See Plate 2.3). Each plot consisted of 16 trees. This gave a total of 160 trees across 10 plots (two plots at each of study areas 2 and 3, and six plots at study area 1). (See map, Plate 2.2).



Plate 2.1 Study area 1 at Loloma field site (August 2002). The transect starts in the centre of the drainage line (Tr1) and extends to the edge of the forest background (Tr6).

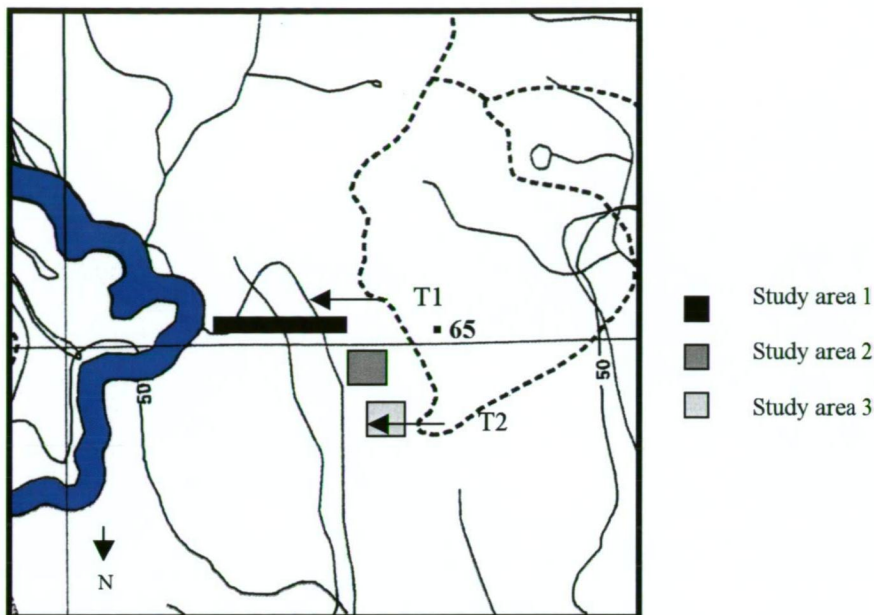


Plate 2.2 Map (1:10000) of three study areas at Loloma, the Little Forester River marks the eastern boundary. Study area 1, represented by the transect, runs from the waterlogged plot (Tr1) at the western end, to the drained plot (Tr6) at the eastern end. The two temperature logger transects are represented by T1 and T2.



Plate 2.3 The waterlogged plot at study area 3 (August 2002). Note the presence of surface water and the associated decline in tree productivity.

Soil descriptions

Soil profiles were dug and described at each of the 10 plots across the three study areas. Soil colour and texture were recorded and soils were classified according

to Forestry Tasmania drainage classes (Laffan 2002). Forestry Tasmania classifies poorly to very poorly drained soils as those with greying to a depth of at least 0.6 m in the profile (Laffan 2002). Soils with grey mottling and/or black castings are classified as imperfectly drained. A small percentage of grey within the profile (2%) defines the soil as moderately well-drained, with well-drained soils presenting no greying.

Microclimate

The incidence of frost events was investigated across the field site to determine whether frost was a compounding factor in reducing productivity in the low-lying and waterlogged areas. TinytagTM temperature loggers, programmed to log air temperature hourly from midday 28 August to midday 10 November 2003, were installed at 25 m intervals along two transects, the first at study area 1 (150 m) and the second at study area 3 (100 m). Each transect of temperature loggers was run from an upslope region of the plantation to the centre of the waterlogged plot at study areas 1 and 3 (Plate 2.2). Each logger was installed within a section of PVC pipe and positioned on a stake 1.3 m above ground level on the outer edge of the northern canopy of a tree. The absolute minimum, mean minimum and mean daily temperatures and the number of frost events (0°C or below at ground level) at each logger point was calculated.

Soil water table and chemistry

Deep (2.5 m) and shallow (0.5 m) soil inspection wells were installed at all plots using a 0.65 m handheld sand auger. Lengths of PVC pipe 50 mm in diameter, that had been slotted to allow water to penetrate the pipe, were dropped into the prepared wells. For shallow wells, 1.5 m lengths of PVC pipe were installed to a depth of 1 m. Coarse sand was filled around the side, to within 0.1 m of the soil surface to allow only subsurface water to enter. The pipes were sealed in position at ground level with bentonite clay to prevent water from cascading down the pipe from the soil surface. For deep wells 3.5 m lengths of PVC pipe were installed. Pipes were slotted to within 0.35 m of the soil surface and filled to this height with coarse sand. The pipe was sealed with bentonite clay 0.35 m from the soil surface. All pipes were capped. Deep water table heights were measured in the

deep wells, and surface (perched) water fluctuations were measured in the shallow wells. Dissolved oxygen (DO), redox potential (Eh) and pH of soil water were measured using a handheld dissolved oxygen – mV – pH – temperature meter (model WP-91, TPS Pty Ltd.). All measurements were conducted at each inspection well (deep and shallow) every 2 months from May 2003 until November 2004.

Tree growth

Tree height, stem basal diameter and crown dimensions (the diameter measured in two directions at right angles at ground level, north/south and east/west) were conducted on all 160 trees at the 10 plots, every 1-2 months from November 2002 to October 2004.

Average tree (stem) volume was calculated at each growth measurement using the equation:

$$\text{Tree (conic) volume (m}^3\text{)} = \frac{1}{3} \times \Pi \times (\text{stem radius})^2 \times \text{tree height (m)}$$

Tree physiology

Physiological measurements were conducted on three trees at the waterlogged and control plot at each of the three sites (total of six plots) in March 2003, April 2003, July 2003, September 2003, December 2003 and May 2004. Mature leaves from the lowest third of the canopy were used to measure water potential in a pressure chamber (PMS Instrument Co., Corvallis, Oregon). These leaves will develop lowered water potentials later than new growth, and as such represent total plant water status.

A portable flourometer (PAM-2000, Heinz Walz GmbH, Efeltrich, Germany) and 2030-B leaf-clip holder was used to measure photosynthetic efficiency (predawn F_v/F_m) on waterlogged and control trees at each site. East facing, mid canopy, healthy fully-expanded leaves were selected for measurement, as these leaves receive sunlight earlier in the day, and are therefore more susceptible to photoinhibition. Three leaves were measured per tree. At the time of F_v/F_m measurement, three leaves per tree were removed and immediately frozen in

liquid nitrogen for future pigment analysis. Samples were transported to Hobart and stored at -20°C.

Midday (between 10.30 am and 12.30 pm) photosynthetic rates and stomatal conductance were measured at a light intensity of 1000 μE , carbon dioxide concentration of 370 μmol and at ambient temperature, using the portable photosynthesis system (LI-COR, Li-6400). Recently fully-expanded leaves in full sun were selected for measurement, as these leaves have maximum photosynthetic rates.

Leaf Pigment Analysis

The protocol used for pigment extraction was based on that of Close et al. (2003). Leaf samples were transferred from the -20 °C freezer to the laboratory on ice and stored in the freezer. Pigment extraction was conducted using 0.2 – 0.3g of leaf discs punched from the three sample leaves using a hole punch. Leaf discs were ground in 10 mL 80% acetone with 25 mg CaCO_3 using a Polytron PT2100 homogeniser. Samples were centrifuged for 10 minutes at 2500 rpm, with the resulting supernatant saved. Pellets were re-extracted using 10 mL 100% acetone. Pigment extracts were stored at -20°C and analysed within 48 hours of extraction.

HPLC analyses were performed using a Waters Alliance 2690 HPLC with a Waters 996 Photodiode Array Detector. The column was an Alltech Allshere ODS-1 5 micron, 250 mm x 4.6 mm fitted with an Alltech Allshere PDS-1 5 micron guard cartridge (7.5 mm x 4.6 mm). The flow rate for all separations was 1.6 mL min⁻¹. All sample injections were 20 μL . The solvents used were; A: Methanol; B: 86.75% Acetonitrile, 9.65% Methanol, 3.6% 0.1M TRIS buffer at pH 8.0 and C: Hexanes. The gradient run was 8% solvent A and 92% solvent B for 3 minutes, then programmed linearly to 85% solvent A and 15% solvent C at 12.5 minutes. The column was re-equilibrated between samples with solvent A for 7 minutes. Data collection was over the range of 250 nm to 700 nm once per second at a resolution of 1.2 nm, and chromatograms were extracted from this at 440 nm, 497 nm and 650 nm. Quantification was based on the 440 peak areas.

Conversion factors were calculated using the absorbance peak area of β -carotene. Conversion factors for peak area to nmol per injection were: violaxanthin (20.72); lutein (27.10); zeaxanthin (26.9); chlorophyll *a* (34.94); chlorophyll *b* (38.53), β -carotene (18.94) (Gilmore and Yamamoto 1991). Antheraxanthin was estimated with the conversion factor for lutein. Neoxanthin was estimated using the conversion factor for violaxanthin (Gilmore and Yamamoto 1991).

Statistical analyses

At Site A, tree physiology (midday photosynthesis maximum, leaf water potential and predawn chlorophyll fluorescence) was analysed by t-test. Leaf pigment chemistry results were analysed using a 2-way ANOVA, using the proc and GLM procedures of SAS 9.1 (SAS Institute Inc., Cary, USA, 2002-2003) to test for the effect of site and waterlogging. Variables were tested for normality and transformed (using log transformations) if required. Final height and volume data was analysed using t-test.

2.2.2 Site B

Site selection

A second field site was established at a plantation outside Geeveston in southern Tasmania (map reference; map sheet Geeveston 4822 1:10000; 48800mE, 22300 mN). This 50 ha site was previously native forest, dominated by *Eucalyptus obliqua*. Its geology was dolerite, with a south-west aspect. The altitude was 300 m a.s.l, with an average annual rainfall of 1200 - 1400 mm. The site was burnt in 1997, windrowed and planted with *E. globulus* in 1998. The site did not receive fertiliser. This site had a number of areas of poor drainage identified prior to plantation establishment.

Plot description

Two study areas were selected at the Geeveston site. At area 1, a waterlogged and a control (drained) plot, each of 10 trees, were established. The control plot was 100 m upslope of the waterlogged plot. Waterlogged plots were identified by the presence of surface water, associated with a severe reduction in tree height.

Study area 2 comprised a 150 m east-west transect, running upslope from waterlogged trees to well-drained and productive trees. Seven plots of 10 trees were established at 25 m intervals along the transect. Understorey co-occurring species included *Leptospermum scoparium*, *Acacia melanoxylon*, *Blechnum* spp., *Spaghnum* spp., *Melaleuca squamea*, *Acacia dealbata*, *Pittosporum bicolor* and *Pomaderris apetala*, indicating waterlogged conditions.

Soil descriptions

Soil profiles were dug to bedrock at 50 m intervals along the transect and soil texture, colour and depth were recorded.

Tree growth

Tree height and basal diameter were measured on all trees between March 2003 and November 2004.

2.3 Results

2.3.1 Site A

Environment

Rainfall

The rainfall of Loloma is represented by the nearby station at Nabowla (Figure 2.1). Peak annual rainfall occurs in winter (June to August). The lowest rainfall occurs in January. The long term mean annual rainfall is 1003 mm.

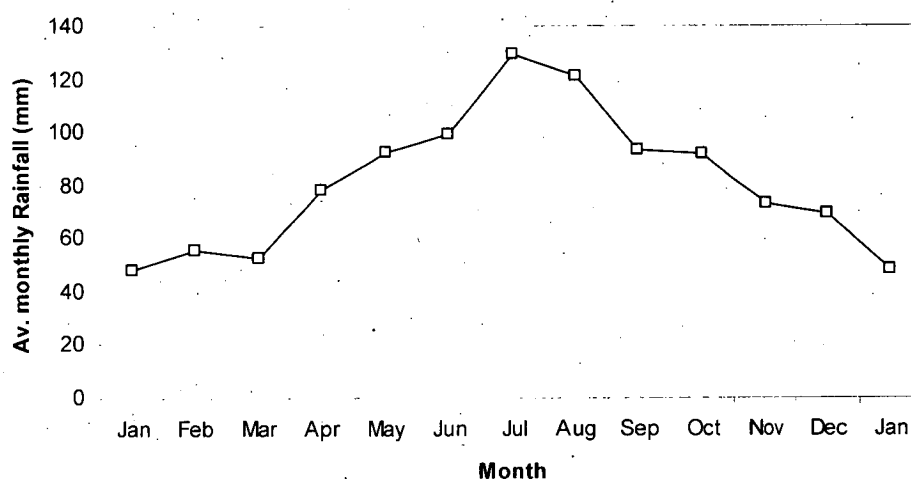


Figure 2.1 Long term (1916 – 2004) mean monthly rainfall for Nabowla (within 1 Km of Loloma).

Temperature

The total number of frost events over the 74 days of measurement (August to November) ranged from 29 at area 1 to 34 at area 2 across the two transects. There were no significant differences in the number of frost events, absolute minimum, mean minimum or mean daily temperature along either of the two transects (Table 2.1). There was no effect of landscape position on temperature.

Table 2.1 Incidence of frost events, absolute minimum temperature, mean minimum temperature and mean daily temperature from tiny tag temperature loggers at transects 1 and 2 at Loloma field site for the 74 days between August 2003 through to November 2003. A frost event was defined as the temperature at or below 0 °C at ground level.

Transect point (distance)	Number of frosts	Absolute minimum (°C)	Mean minimum (°C)	Mean daily temperature (°C)
Transect 1 (study area 1)				
1 (0 m) upslope	33	-5.7	5.1	10.5
2 (25 m)	33	-5.5	5.3	10.5
3 (50 m)	33	-5.3	5.5	10.5
4 (75 m)	33	-5.5	5	10.2
5 (100 m)	34	-5.7	5	10.1
6 (125 m)	34	-5.9	4.9	10.4
7 (150 m)	30	-5.4	5.2	10.7
8 (175 m) waterlogged	29	-5.5	5	10.2
Transect 2 (study area 3)				
1 (0 m) upslope	32	-4.9	5.5	10.6
2 (25 m)	32	-5.1	5.2	10.2
3 (50 m)	34	-5.4	5.6	10.6
4 (75 m)	34	-5.7	5.4	10.5
5 (100 m) waterlogged	34	-6.2	4.9	10.5

Physical factors of soil

Soil descriptions

Soils at each waterlogged plot were predominantly grey to a depth of at least 0.6 m (Table 2.2). Along the transect at study area 1, the soil became progressively better drained from the waterlogged plot (Tr1) through to the drained plot (Tr6).

The degree of soil greying decreased from Tr1 and Tr2, plots classified as poorly drained, to Tr3 and Tr4 classified as imperfectly drained, to Tr5 and Tr6 which were moderately well drained plots (Table 2.2). There was a strong relationship between the degree of soil greying, indicative of long term waterlogging, and tree productivity (in height and stem conic volume).

Greying of the profile at waterlogged plots at study areas 2 and 3 was evident within the top 0.6 – 0.7 m of soil, with grey mottling to a depth of up to 1.5 m. Drained plots had no substantial greying (some mottling at study area 2) (Table 2.2).

Table 2.2 Soil descriptions, drainage class and mean tree height of the six transect plots (Tr1 to Tr6) at area 1 and the drained and waterlogged plots at areas 2 and 3 in November 2002.

Plot	Soil description	Drainage class	Mean tree height (cm)
Study area 1			
Waterlogged (Tr1)	<u>A horizon</u> – Dark brown sandy loam 0-10 cm. <u>B1g horizon</u> – Grey clay to 10-70 cm. Gradual boundary. <u>B2 horizon</u> – Yellow silty-clay with grey mottling to 70-100+ cm.	Poorly drained	101
Tr2	<u>A horizon</u> – Brown loam 0-30 cm. Sharp boundary. <u>Bg horizon</u> – Grey clay to a depth of 70 cm.	Poorly drained	118
Tr3	<u>A horizon</u> – Friable loam 0-15 cm. Sharp boundary. <u>B1g horizon</u> – Grey clay to 15-20 cm. Gradual boundary. <u>B2 horizon</u> – Yellow grey-mottled sandy loam 20-100+ cm.	Imperfectly drained	157
Tr4	<u>A horizon</u> – Organic loam topsoil 0-20 cm. <u>B1g horizon</u> – Grey-mottled friable loam 20-60 cm. Gradual boundary. <u>B2 horizon</u> – Yellow silty-clay 60-200 cm.	Imperfectly drained	157
Tr5	<u>A horizon</u> – Dark brown loam 0-10 cm. <u>B horizon</u> – Brown friable clay loam 10-40 cm with black castings. Gradual boundary. <u>BC horizon</u> – Yellow clay 40-250 cm, becoming progressively drier with depth	Well-drained	171
Drained (Tr 6)	<u>A horizon</u> – Dark brown loam 0-20 cm. <u>B horizon</u> – Dark yellow clay to 250 cm, becoming drier and more friable with depth.	Well-drained	245
Study area 2			
Waterlogged	<u>A horizon</u> – Brown loam 0-10 cm. <u>Bg horizon</u> – Grey clay loam 10-120 cm.	Poorly drained	112
Drained	<u>A horizon</u> – Brown loam 0-10 cm. <u>B1g horizon</u> – Grey-mottled clay to 10-30 cm. Gradual boundary. <u>B2 horizon</u> – Yellow clay 30-220 cm, becoming more friable with depth.	Moderate to well- drained	228
Study area 3			
Waterlogged	<u>A horizon</u> – Loam 0-15 cm. <u>B1g horizon</u> – Grey clay to 65 cm. Gradual boundary. <u>B2g horizon</u> – Yellow clay with grey-mottling 65-150 cm.	Poorly drained	127
Drained	<u>A horizon</u> – Loam 0-10 cm. <u>B1 horizon</u> – Friable clay-loam to 10-50 cm. Gradual boundary. <u>B2 horizon</u> – Yellow friable clay to 50-250 cm.	Well-drained	226

Waterlogging

No water was present in the inspection wells until the beginning of June in 2003 (Figures 2.2, 2.3 and 2.4). Water levels rose to the soil surface during June.

In the drained plots at area 1 (Tr6) the water table remained 0.5 m below the soil surface in deep wells and 0.3 m below the soil surface in shallow wells throughout June and July 2003.

At the waterlogged plot at area 1 (Tr1), the water table rose to the soil surface during June in both the deep and shallow wells and remained at the soil surface for approximately three months (Figure 2.2). Water was present within 1.5 m of the soil surface throughout the 2003/2004 summer at waterlogged plots, rising to re-saturate the soil profile in July 2004.

At area 2 the drained plots had water at the soil surface in July, in both the deep and shallow wells. Waterlogging was more severe at waterlogged plots, where water tables were higher for longer. In deep wells at area 2, water tables at the waterlogged plots did not recede to below 1.7 m throughout the measurement period of June 2003 to October 2004.

At area 3, the waterlogged and drained plots had similar water level fluctuations throughout the measurement period (Figure 2.4). Water was present at the soil surface from July to August. The shallow water table receded more rapidly than that in the deep wells. Throughout the 2003/2004 summer (December to March) the water table was below the minimum measurable height in deep (-3.0 m) and shallow (-1.0 m) wells.

There was a consistency in the initiation and length of the waterlogging period between 2003 and 2004 at areas 2 and 3. The only difference was in the length of waterlogging, which was shorter in 2004. There was an increase in the duration and severity of waterlogging in control plots from sites 1 to 3. Therefore, at site 3 there was little difference in the water table height between waterlogged and control plots.

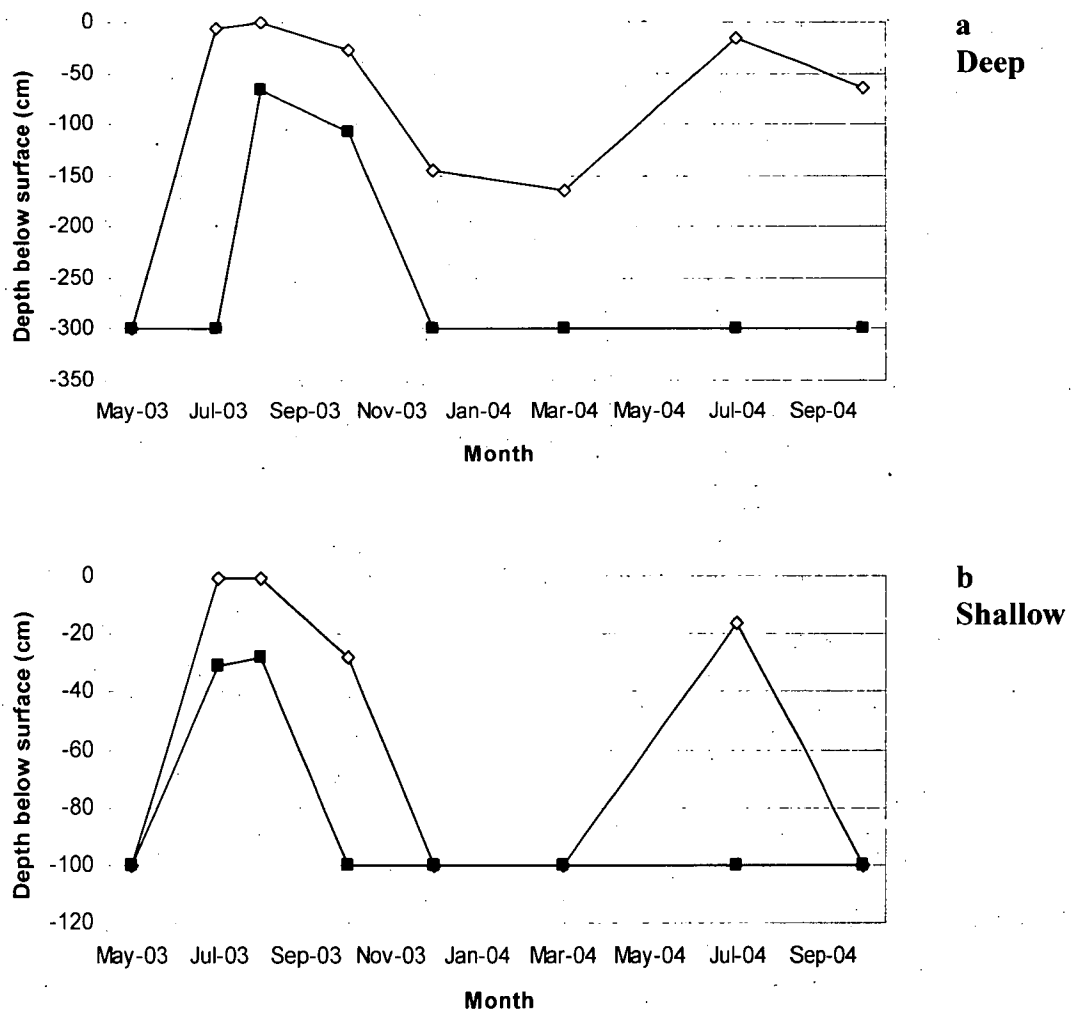


Figure 2.2 The water table depth of deep (a) and shallow (b) wells at waterlogged \diamond and control plots \blacksquare in study area 1.

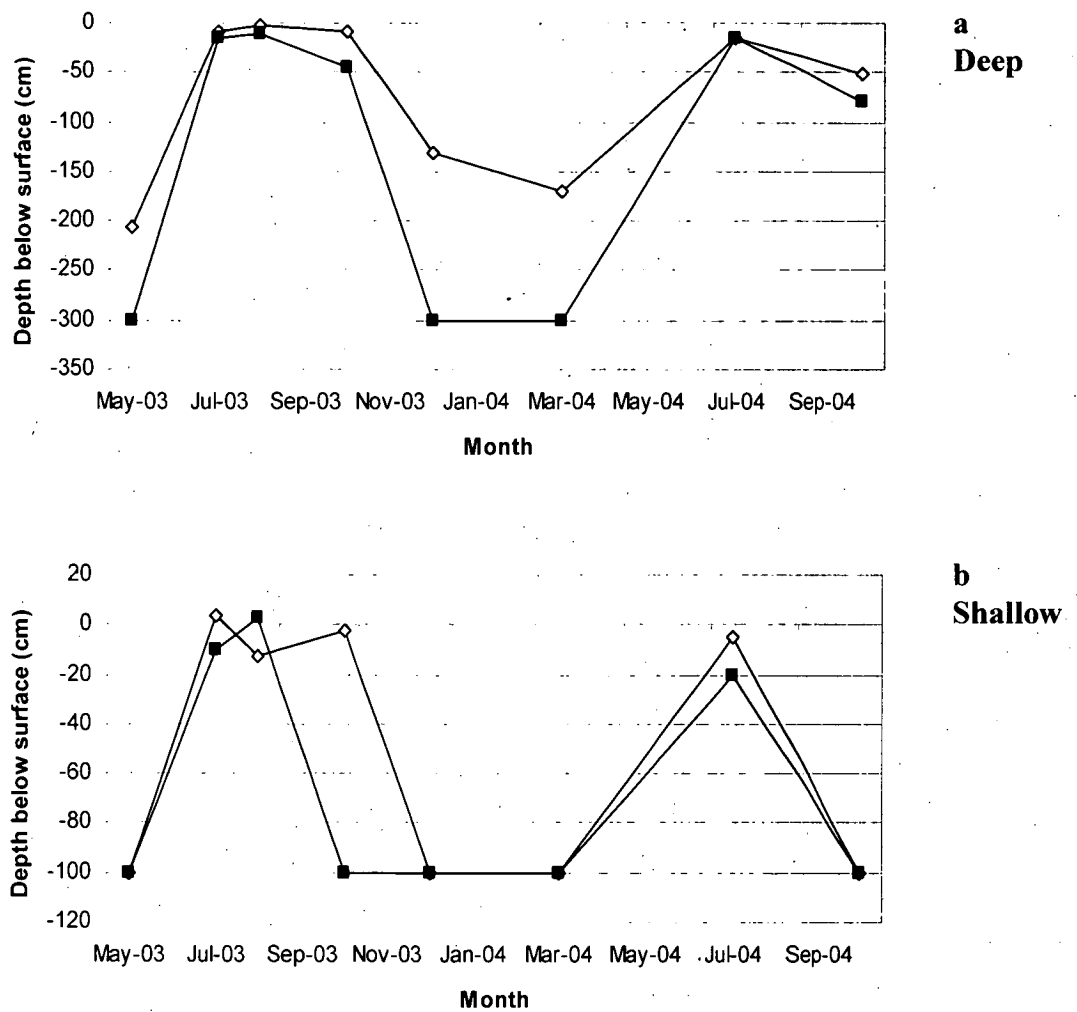


Figure 2.3 The water table depth of deep (a) and shallow (b) wells at waterlogged \diamond and control plots \blacksquare in study area 2.

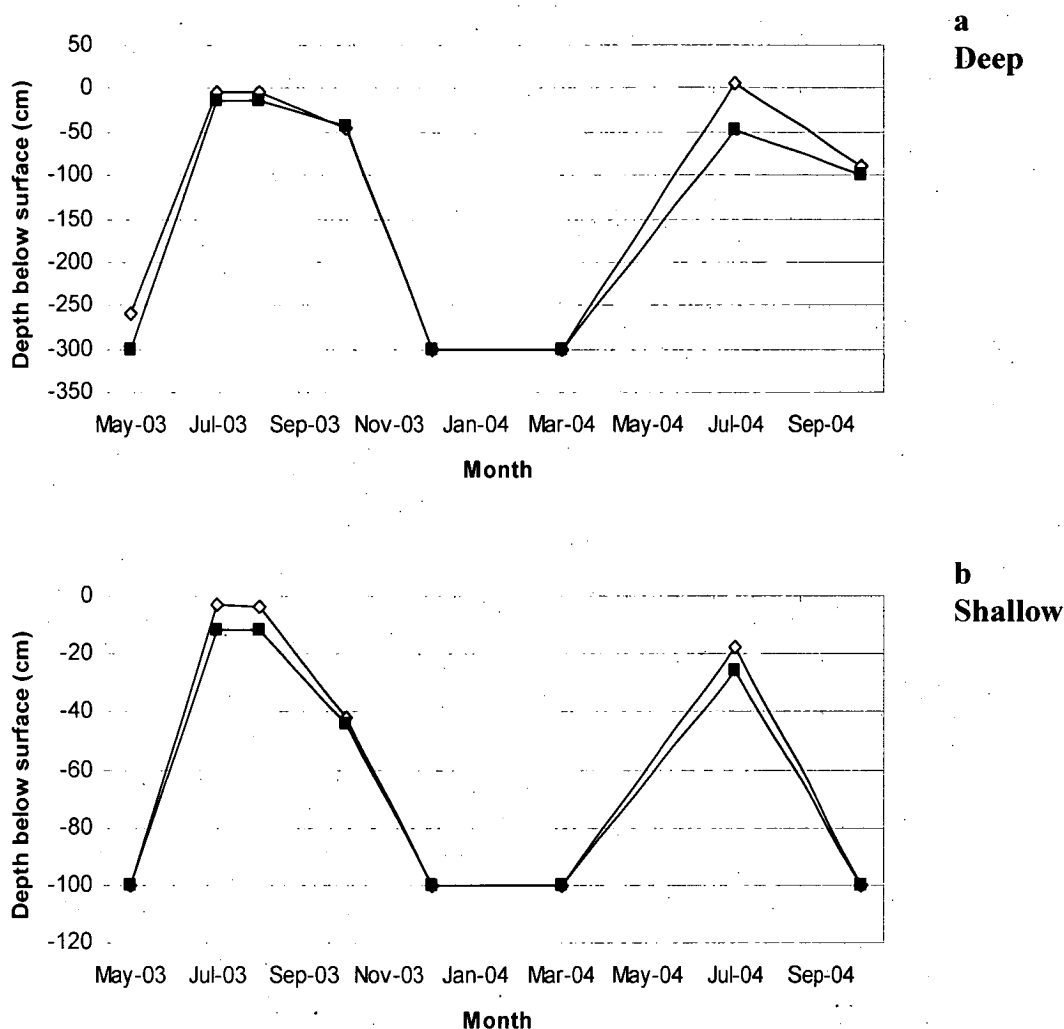


Figure 2.4 The water table depth of deep (a) and shallow (b) wells at waterlogged \diamond and control plots \blacksquare in study area 3.

Water chemistry

In August, after the soil had been saturated for almost three months, the dissolved oxygen (DO) of the soil solution had fallen in all waterlogged plots to approximately 2 ppm, and was consistently lower than that of drained plots. The DO of all wells generally rose between August and October (Table 2.3). There was no consistent difference in DO between the deep and shallow wells. In August, waterlogged plots at areas 2 and 3 had significantly lower pH values than at the drained plots.

There was no significant difference in pH in October (Table 2.3).

The redox potential (Eh) was not significantly different between deep and shallow wells in August 2003. At areas 2 and 3, drained plots had lower Eh values than

waterlogged plots. In October, shallow wells had lower redox values than drained wells across all areas (Table 2.3).

Table 2.3 Soil solution pH, dissolved oxygen (DO) (ppm) and redox potential (Eh) (mV) sampled from deep and shallow wells during August and October 2003.

Plot	pH		DO (ppm)		Eh (mV)	
	Deep	Shallow	Deep	Shallow	Deep	Shallow
August 2003						
1 waterlogged	6.22	5.86	1.91	1.27	151	142.8
1 drained	4.65	4.83	4.5	2.2	166.1	176.5
2 waterlogged	4.33	5.59	2.04	3.96	255.3	206.4
2 drained	5.36	4.89	5.03	1.87	222.4	226.6
3 waterlogged	5.14	5.11	2.1	1.68	222.2	219.1
3 drained	6.36	6.71	5.95	1.33	198	172.9
October 2003						
1 waterlogged	5.28	6.09	4.15	0.2	139.5	72
1 drained						
2 waterlogged	5.2	5.8	2.64	3.53	200.9	161.1
2 drained	4.9		6.25		162.9	
3 waterlogged	5.13	5.13	1.12	0.26	201	137.7
3 drained	5.41	5.41	6.63	5.17	191	163.5

Growth

Tree height

All trees were the same height at planting in August 2001 (approximately 20 cm). The difference in growth between waterlogged and drained trees during the first year resulted in a large difference in tree height by November 2002. The relative growth rate of trees at area 1 over the initial 12 months was 2.25 m year^{-1} in drained trees and 1.44 m year^{-1} in waterlogged trees. This difference in relative growth rate resulted in a 1.45 m difference in mean tree height after one year's growth (Figure 2.5).

The difference in mean tree height between drained and waterlogged trees increased during the subsequent 2 years (2.06 m at the end of 2003, and 2.68 m at the end of 2004). Despite an increasing difference in tree height between waterlogged and drained trees over the first three years of plantation growth, the relative growth rate of waterlogged trees was higher than drained trees in years 2 and 3 (1.03 m year^{-1} compared to 0.85 m year^{-1} at year 2 and 0.57 m year^{-1} compared to 0.44 m year^{-1} at year 3).

There was also a strong seasonal effect on tree growth, with high rates of growth during summer/early autumn (November to April) and low rates during the winter months.

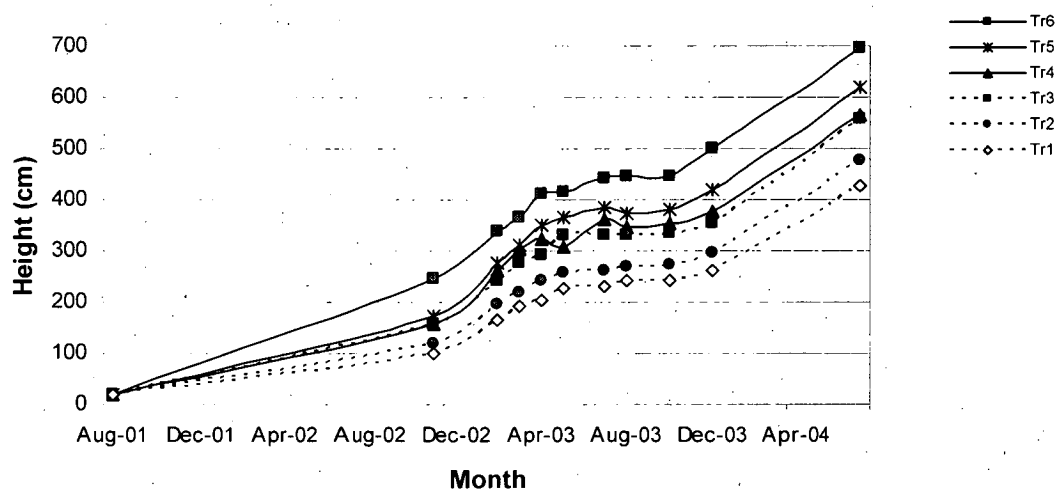


Figure 2.5 Mean tree height (cm) at study area 1, on a transect from waterlogged trees (plot Tr 1) through to drained trees (plot Tr 6). The initial average planting height of 20 cm (planted out in August 2001) has been added to the figure.

At study areas 2 and 3 trees displayed the same seasonal pattern of growth observed at area 1, with high growth rates during summer. Waterlogged trees at area 2 exhibited smaller variations in seasonal growth than drained trees (Figures 2.6). There were large differences in tree height between waterlogged and drained trees, consistent with that of area 1. After three years growth, the difference in mean tree height between drained and waterlogged trees was 3.64 m at area 2 and 2.13 m at area 3 (Figures 2.6 and 2.7).

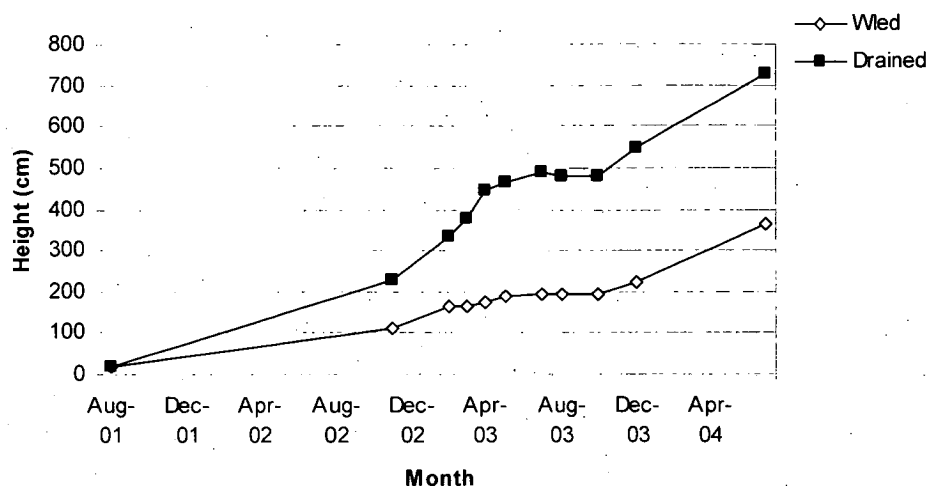


Figure 2.6 Average tree height (cm) of waterlogged (Wled) and drained plots at study area 2. The initial average planting height of 20 cm (planted out in August 2001) has been added to the figure.

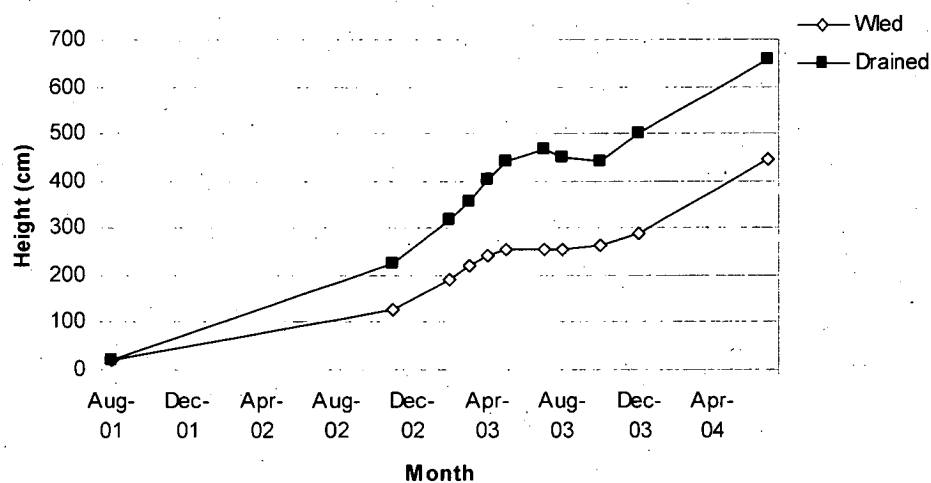


Figure 2.7 Average tree height (cm) of waterlogged (Wled) and drained plots at study area 3. The initial average planting height of 20 cm (planted out in August 2001) has been added to the figure.

Tree volume

Trees in drained plots had a higher average tree volume than waterlogged plots (up to 850 % at area 2) by the age of 3 years. The difference in tree conic volume between waterlogged and drained plots continued to increase over the 18 month period of measurements. In November 2002, after only 15 months growth at the

three study areas, waterlogged trees had a substantially smaller mean tree volume than drained trees (Figures 2.8, 2.9 and 2.10).

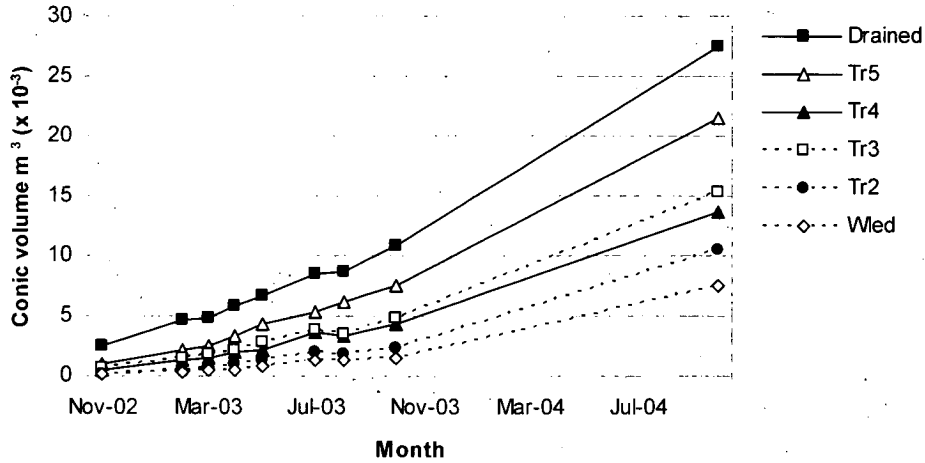


Figure 2.8 Average conic volume of the stem of trees at Loloma field site study area 1 represented by the transect plots (Tr1 – waterlogged through to Tr6 – drained) from November 2002 to October 2004.

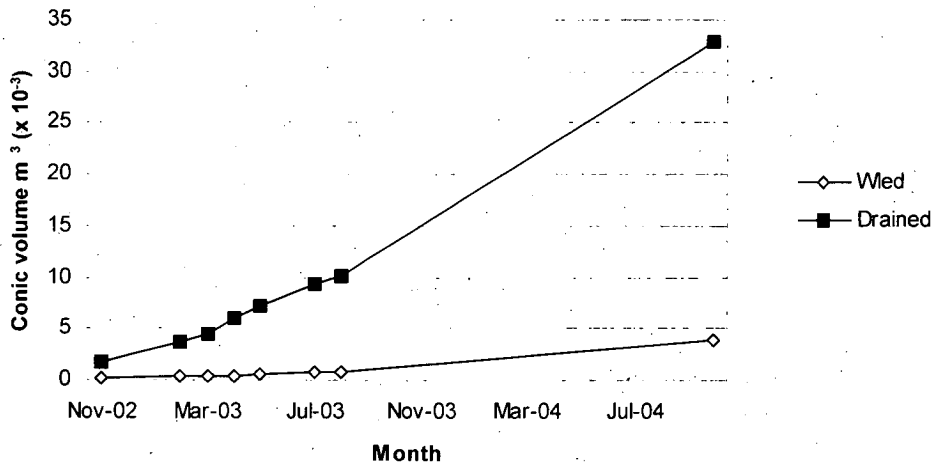


Figure 2.9 Average conic volume of the stem of trees at Loloma field site study area 2 from November 2002 to October 2004.

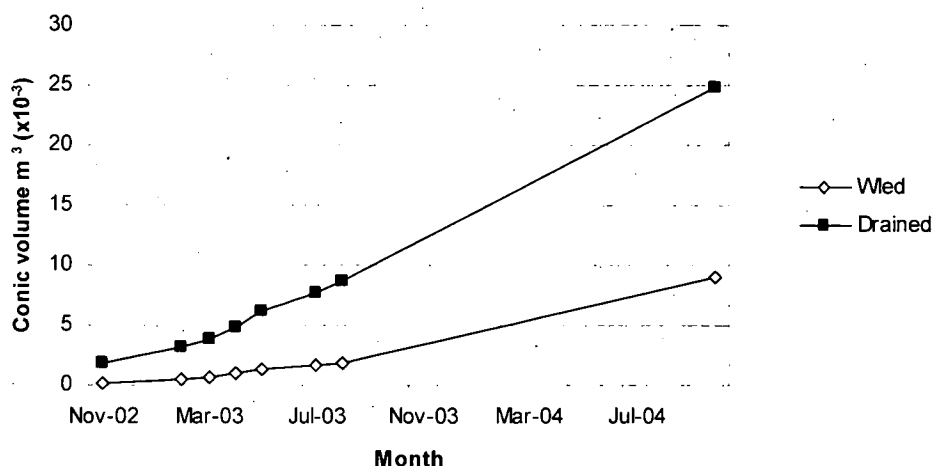


Figure 2.10 Average conic volume of the stem of trees at Loloma field site study area 3 from November 2002 to October 2004.

Physiology

Area 1 (transect plots Tr1 – Tr6)

Stomatal conductance results were very similar to photosynthetic rate results, as is typical in this species, and as such only midday measurements of photosynthetic rate are presented.

There was no significant difference in net photosynthesis in March 2003 between waterlogged (Tr1) and drained trees (Tr6) (Figure 2.11). There was a significant increase ($P < 0.0001$) in photosynthetic efficiency (F_v/F_m) across the transect, with waterlogged trees exhibiting low F_v/F_m (< 0.55), indicating photoinhibition (Figure 2.11b). The predawn leaf water potential was significantly lower ($P < 0.01$) at the drained plot (-0.9 MPa) (Tr6) than the waterlogged plot (-0.4 MPa) (Figure 2.11c).

In July 2003, there was a reversal of trends in photosynthesis relative to March results. The net photosynthetic rate of waterlogged trees had fallen to $< 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2.12a). Photosynthetic rates increased across the transect with drained trees performing best with a rate of $14 \mu\text{mol m}^{-2} \text{s}^{-1}$. The F_v/F_m of all trees had returned to above 0.8 with no variation between waterlogged and drained trees across the experimental transect (Figure 2.12b). Leaf water potential of

waterlogged trees was generally lower ($P = 0.0001$) than that of drained trees (Figure 2.12c).

In December 2003 there was no significant differences in the photosynthetic performance, F_v/F_m or predawn water potential associated with waterlogging or positions along the transect (Appendix 1).

Predawn chlorophyll fluorescence measurements were conducted at study area 1 in May 2004. There were no significant differences between waterlogged and drained plots (Appendix 2).

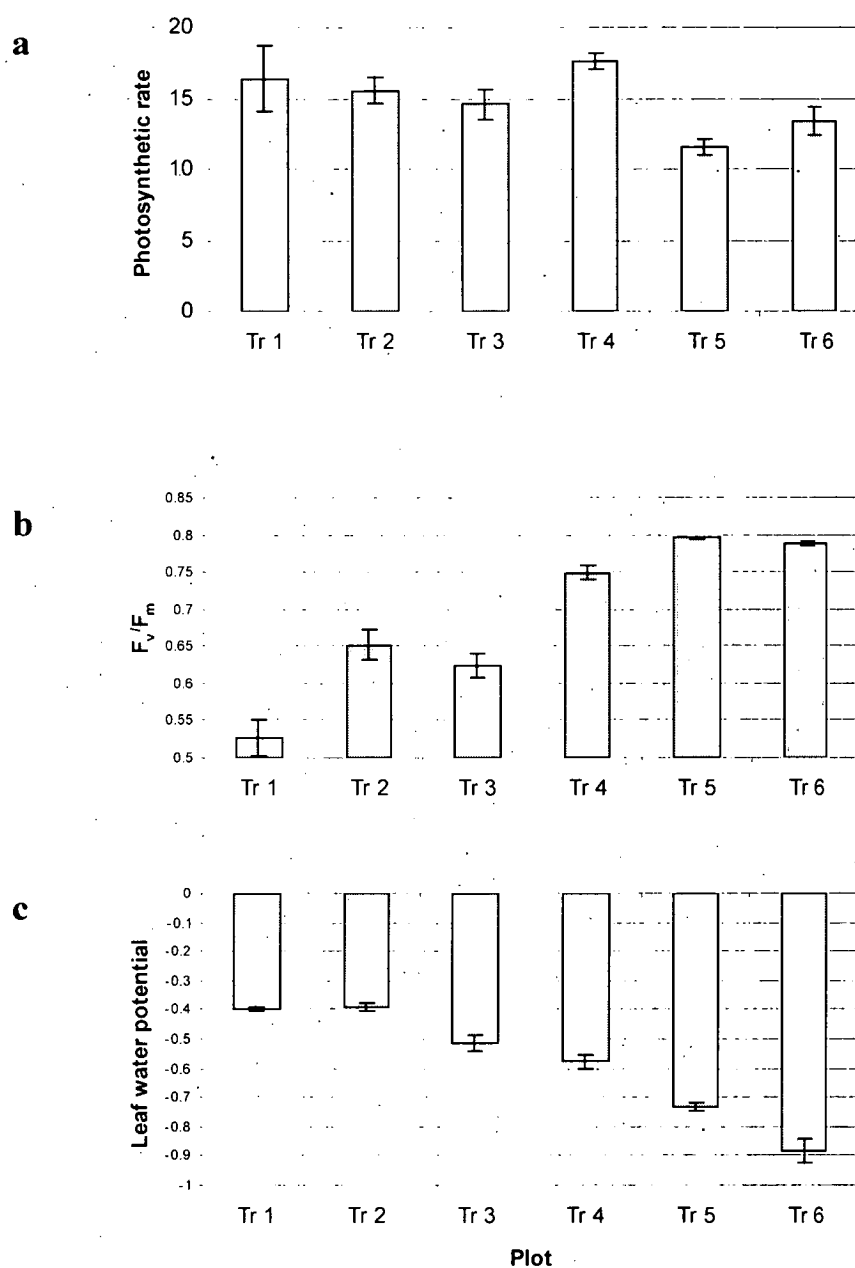


Figure 2.11 (a) Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (b) Photosynthetic efficiency (predawn F_v/F_m) and (c) Predawn leaf water potential (MPa) of plots along the transect at area 1 from waterlogged trees (plot Tr1) to drained trees (plot Tr6) in March 2003. Bars represent standard error.

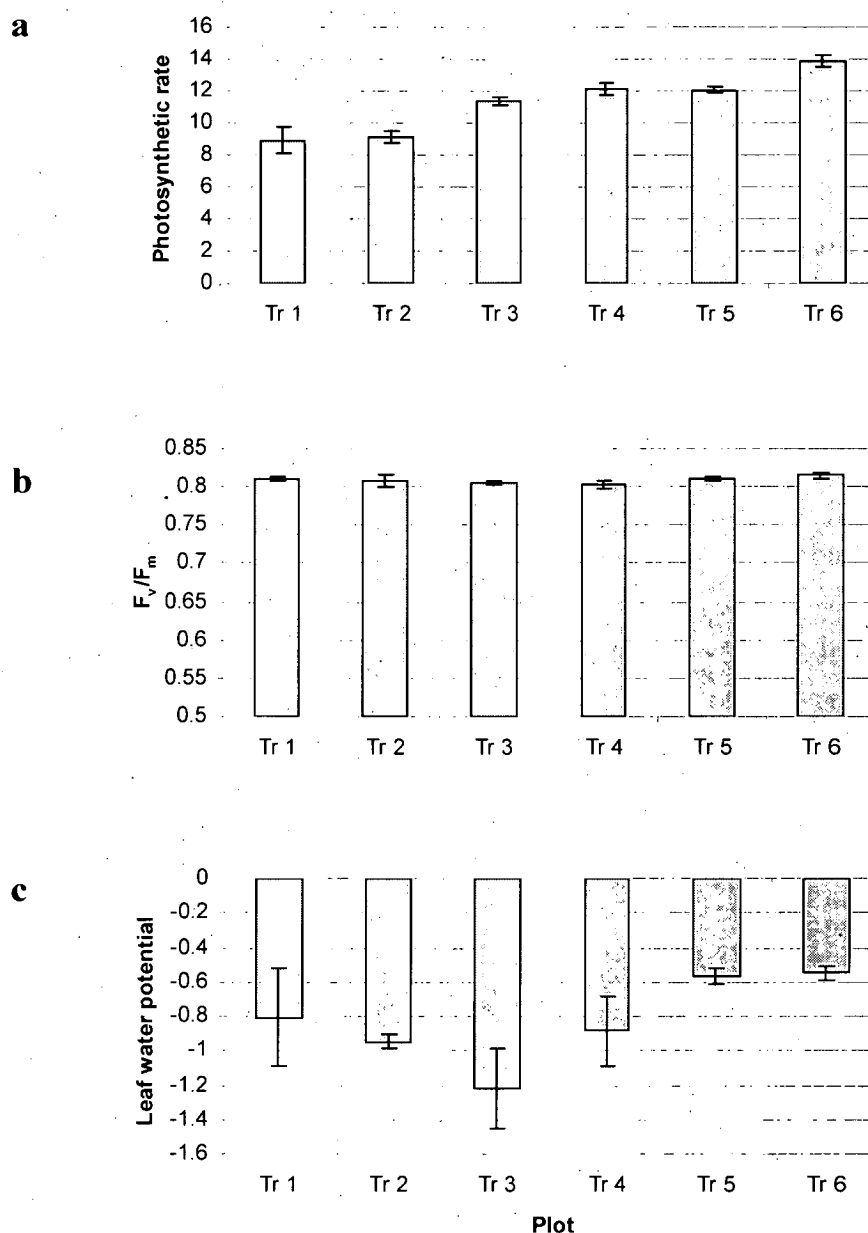


Figure 2.12 (a) Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (b) Photosynthetic efficiency (predawn F_v/F_m) and (c) Predawn leaf water potential (MPa) of plots along the transect at area 1 from waterlogged trees (plot Tr1) to drained trees (plot Tr6) in July 2003. Bars represent standard error.

Comparison of areas 1, 2 and 3

Area 1 is represented by the waterlogged plot (Tr 1) and drained plot (Tr 6) of the transect, as described previously. Areas 2 and 3 have waterlogged and drained plots.

In March there were no significant differences in the photosynthetic performance between waterlogged and drained plots (Figure 2.13a). The F_v/F_m was similar for drained and waterlogged plots at study areas 2 and 3 (Figure 2.13b), but different at area 1. Leaf water potential results were similar for the three areas, with drained plots having lower water potentials than waterlogged plots (Figure 2.13c).

In July 2003, photosynthetic rates were similar across all sites, with waterlogged plots exhibiting a significantly lower ($P < 0.001$) midday maximum than drained plots (Figure 2.14a). There was insufficient data on predawn F_v/F_m , with areas 1 and 2 exhibiting different patterns (Figure 2.14b). There were differences between the predawn leaf water potentials of drained and waterlogged plots at areas 1 and 2 (Figure 2.14c). By midday, all trees had a lower leaf water potential than at dawn, and no significant differences between drained and waterlogged plots at areas 2 and 3 (Figure 2.15). At area 1, drained trees had a significantly lower ($P < 0.001$) water potential than waterlogged trees.

The predawn F_v/F_m ratios of waterlogged plots were significantly lower ($P < 0.05$) than drained plots across all three areas in September 2003 (Figure 2.16), but all values were above those considered indicative of photoinhibition.

In December 2003 the photosynthetic rates were higher in drained than waterlogged plots across all sites (Figure 2.17a). There was no difference between waterlogged and drained trees in photosynthetic efficiency and predawn leaf water potential (Figure 2.17 b and c).

In May 2004 the midday photosynthetic rate of waterlogged and drained plots did not differ at study area 1 or 3. Waterlogged trees had lower midday maximum than drained trees at study area 2 (Figure 2.18).

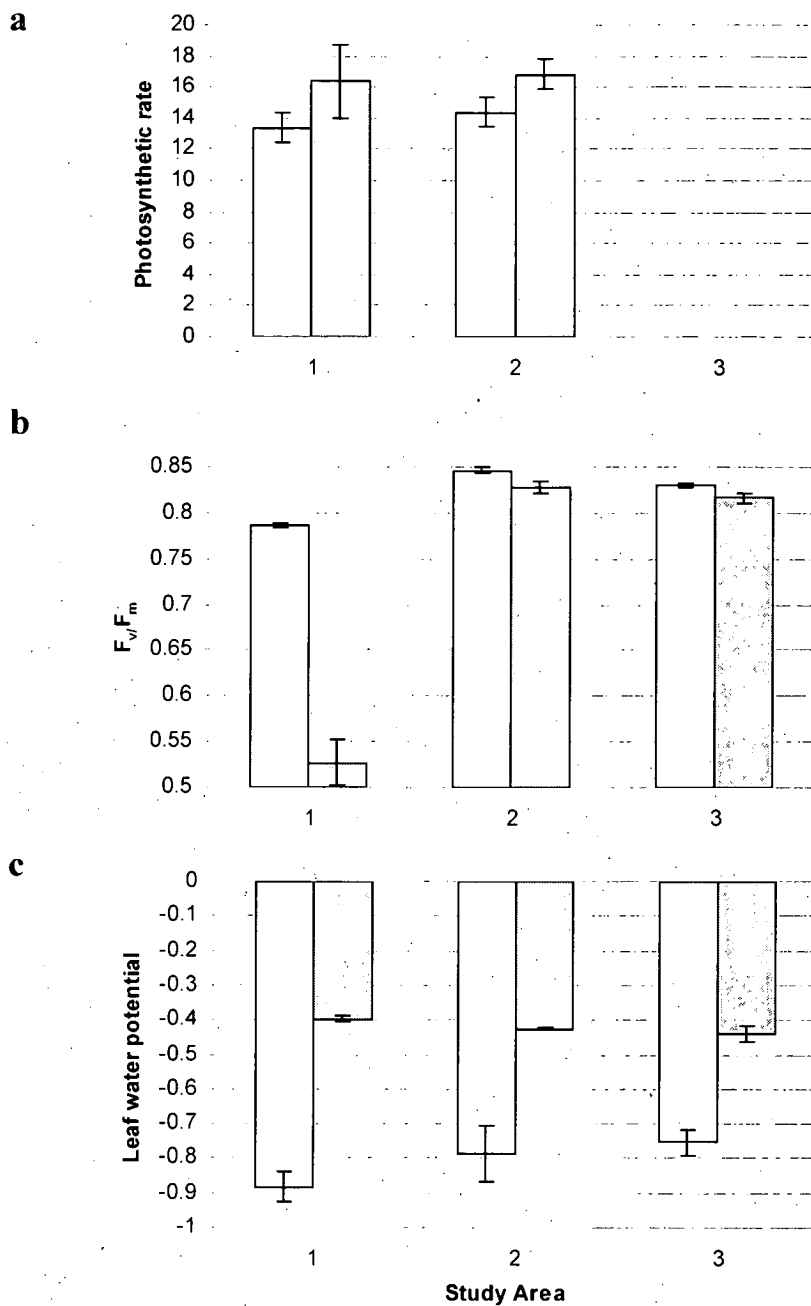


Figure 2.13 (a) Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (b) Photosynthetic efficiency (predawn F_v/F_m) and (c) Predawn leaf water potential (MPa) of drained \square and waterlogged \blacksquare plots at study areas 1, 2 and 3 at Loloma field site in March 2003. Bars represent standard error.

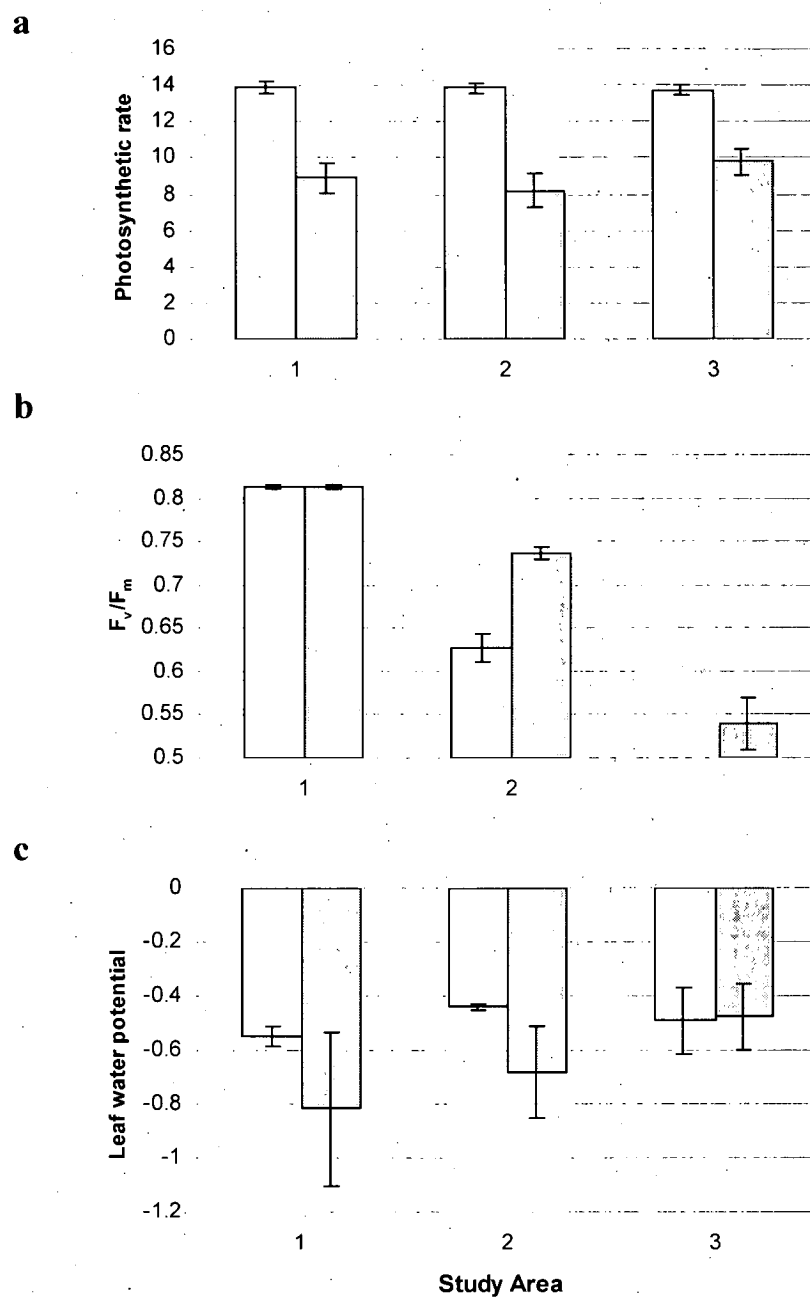


Figure 2.14 (a) Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (b) Photosynthetic efficiency (predawn F_v/F_m) and (c) Predawn leaf water potential (MPa) of drained □ and waterlogged ■ plots at study areas 1, 2 and 3 at Loloma field site in July 2003. Bars represent standard error.

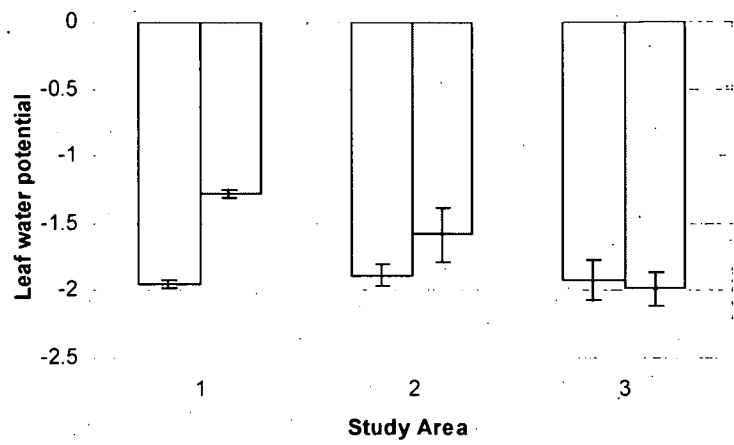


Figure 2.15 Midday leaf water potential (MPa) of drained and waterlogged plots at sites 1, 2 and 3 at Loloma field site in July 2003. Bars represent standard error.

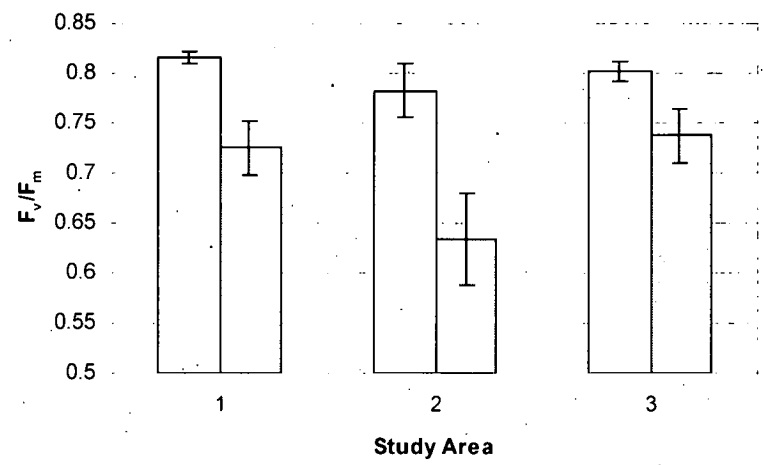


Figure 2.16 Predawn F_v/F_m of drained and waterlogged plots at sites 1, 2 and 3 at Loloma field site in September 2003. Bars represent standard error.

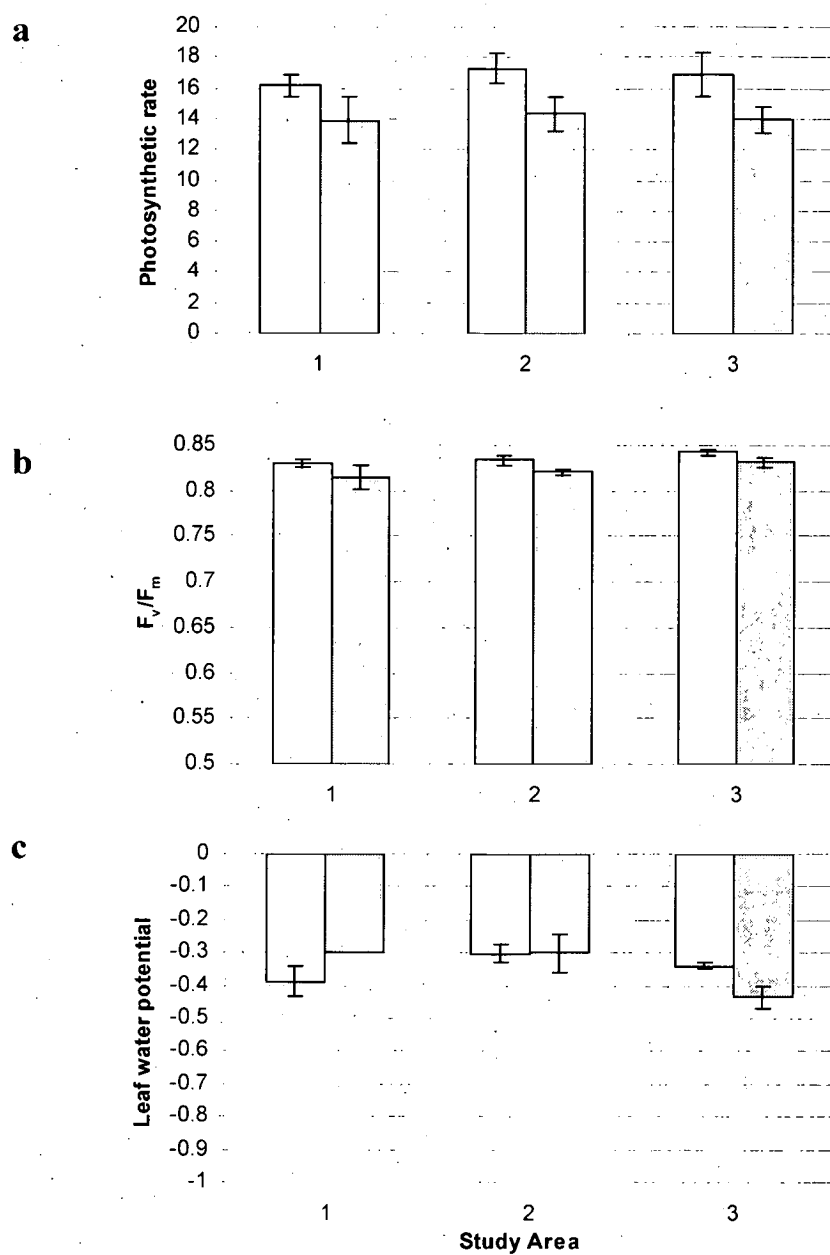


Figure 2.17 (a) Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (b) Photosynthetic efficiency (predawn F_v/F_m) and (c) Predawn leaf water potential (MPa) of drained \square and waterlogged \blacksquare plots at sites 1, 2 and 3 at northern field site in December 2003. Bars represent standard error.

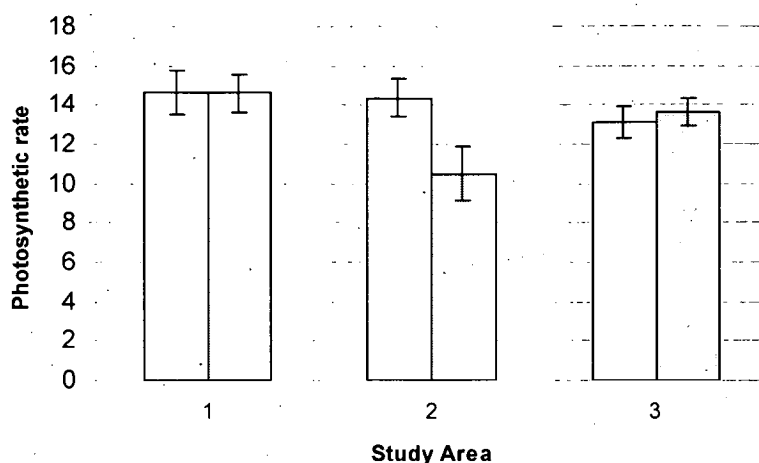


Figure 2.18 Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of drained \square and waterlogged \blacksquare plots at areas 1, 2 and 3 at the northern field site in May 2004. Bars represent standard error.

Pigment analysis

In March, drained trees had a significantly higher concentration of foliar neoxanthin ($P < 0.001$), lutein ($P = 0.0001$), β -carotene ($P = 0.0006$), total xanthophylls pool size ($P = 0.011$) and chlorophyll ($P = 0.0001$) than waterlogged trees (Figures 2.19, 2.20, 2.21, 2.22 and 2.24). However, this was not evident in the xanthophyll conversion ratio (az/vaz) (Figure 2.23).

In July 2003, there were no significant effects of waterlogging on pigment concentrations (data not shown). At the end of the winter season (peak waterlogging season) in September, there was a significant effect of waterlogging on the xanthophyll conversion ratio ($P = 0.0167$). Waterlogged plots had significantly higher active xanthophyll pigment engagement than drained plots at study areas 1 and 2, but not 3 (Figure 2.25).

The peak in neoxanthin, lutein, β -carotene and chlorophyll concentrations occurred in March for drained plots and July for waterlogged plots across all study areas. The peak in total xanthophyll pool occurred during July – September, with no consistent response from drained or waterlogged plots across the three study areas. Waterlogged plots did not show a significant depression in

chlorophyll concentration during the waterlogging season. Chlorophyll concentrations were at their lowest in December 2003.

There was no significant effect of waterlogging or area on pigment chemistry in December.

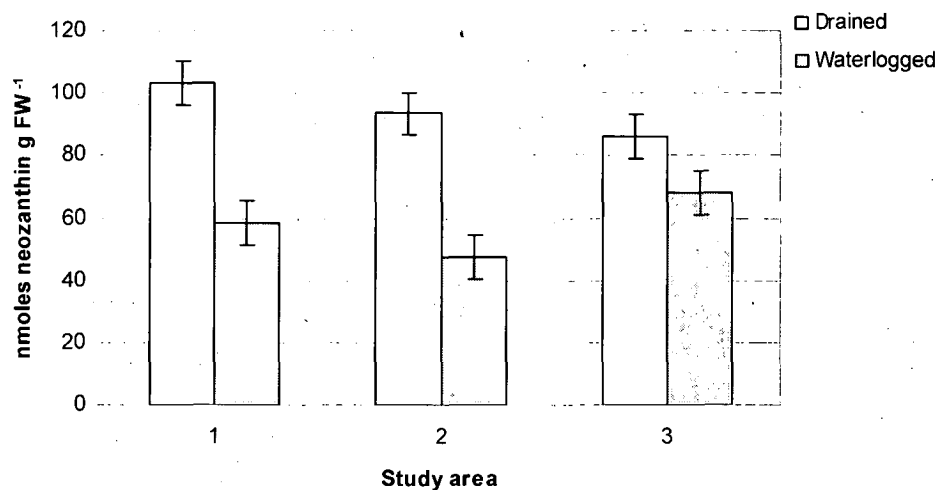


Figure 2.19 nmoles neoxanthin per gram leaf fresh weight (g FW⁻¹) at waterlogged and drained plots across all three study areas in March 2003. Bars represent standard error.

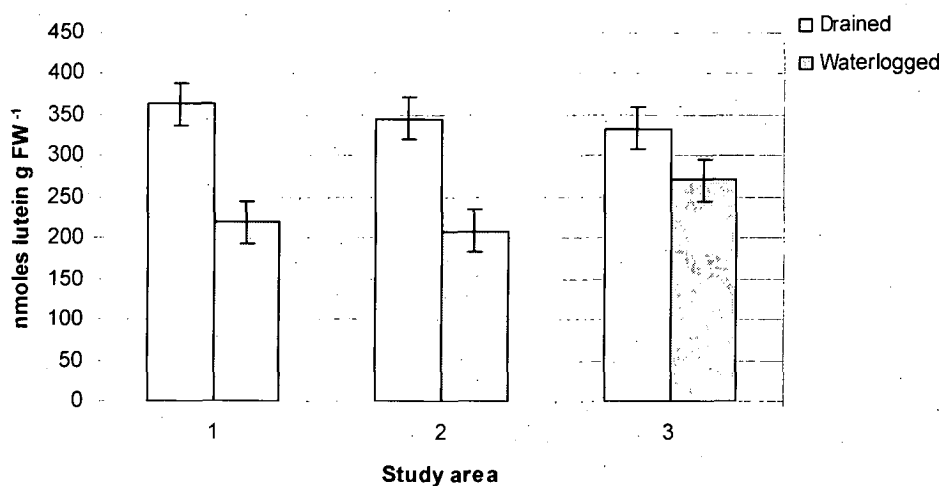


Figure 2.20 nmoles lutein per gram leaf fresh weight (g FW⁻¹) at waterlogged and drained plots across all three study areas in March 2003. Bars represent standard error.

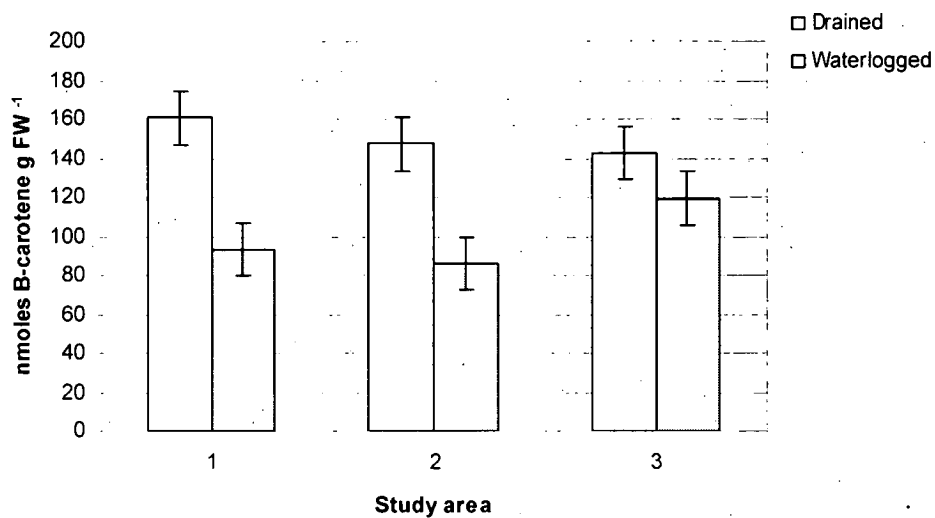


Figure 2.21 nmoles β -carotene per gram leaf fresh weight (g FW⁻¹) at waterlogged and drained plots across all three study areas in March 2003. Bars represent standard error.

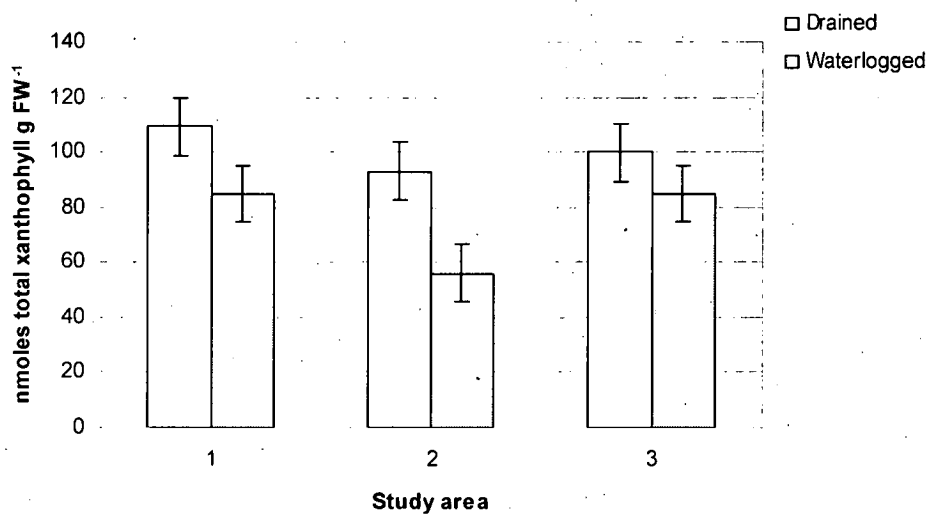


Figure 2.22 nmoles total xanthophyll pigment pool per gram leaf fresh weight (g FW⁻¹) at waterlogged and drained plots across all three study areas in March 2003. Bars represent standard error.

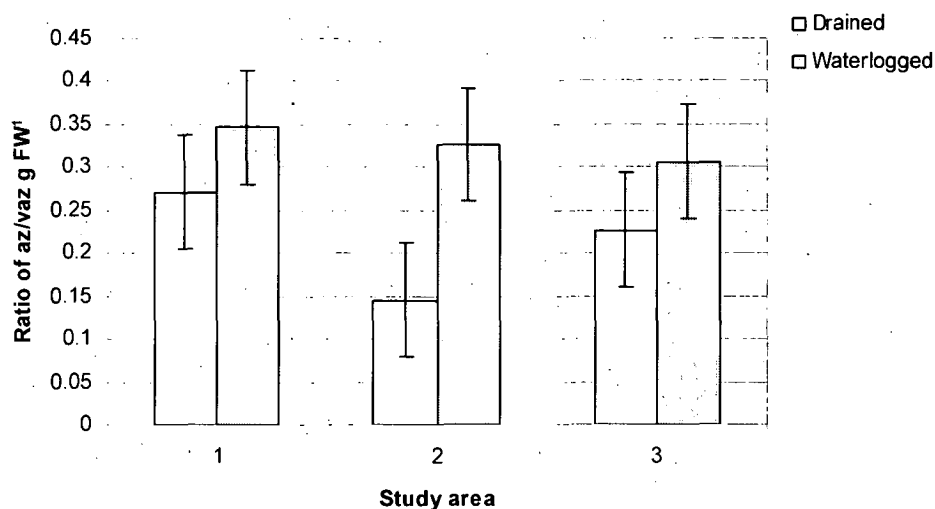


Figure 2.23 Ratio of az/vaz (ratio of active to total xanthophyll pigments) per gram leaf fresh weight (g FW⁻¹) at waterlogged and drained plots across all three study areas in March 2003. Bars represent standard error.

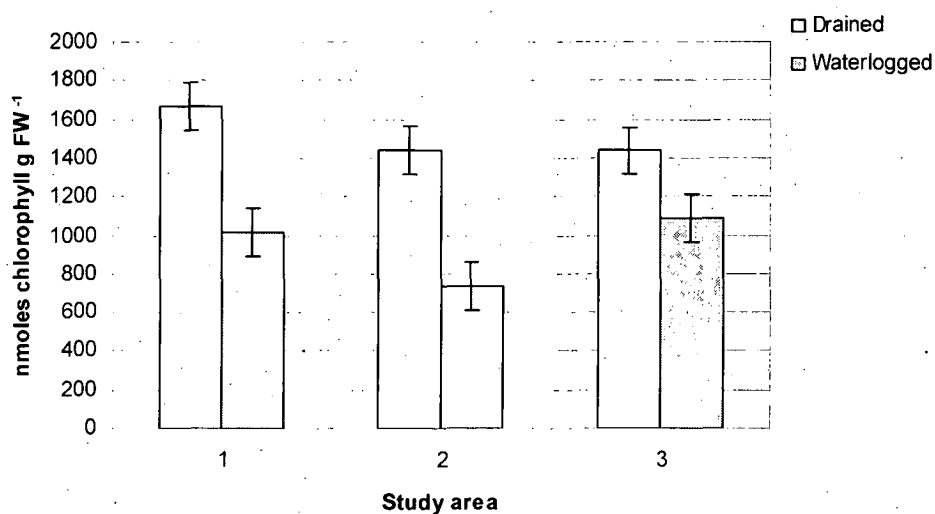


Figure 2.24 nmoles chlorophyll (a and b) per gram leaf fresh weight (g FW⁻¹) at waterlogged and drained plots across all three study areas in March 2003. Bars represent standard error.

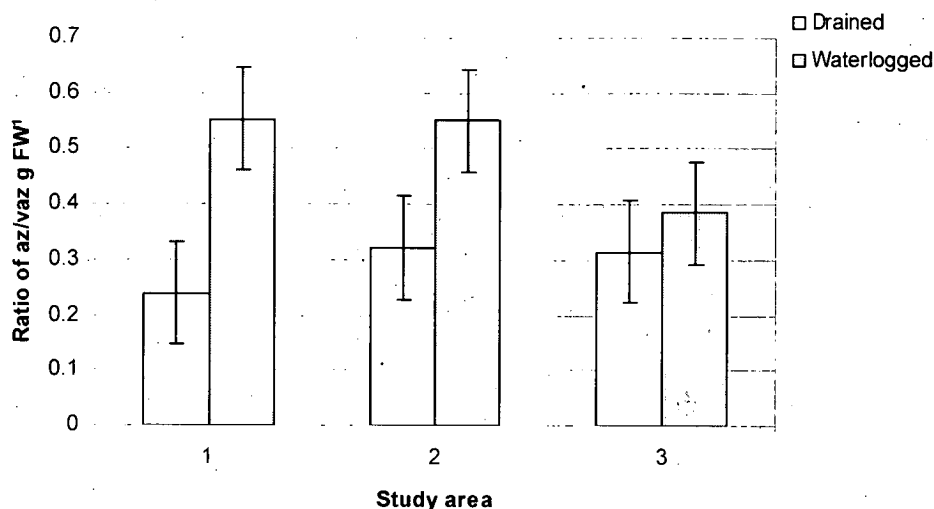


Figure 2.25 Ratio of az/vaz (ratio of active to total xanthophyll pigments) per gram leaf fresh weight (g FW^{-1}) at waterlogged and drained plots across all three study areas in September 2003. Bars represent standard error.

2.3.2 Site B

Environment

Rainfall

The rainfall pattern of Geeveston is represented by the nearby station at Fourfoot Road (Figure 2.26). The annual peak in rainfall occurs in July and high rainfall throughout the winter and spring months. The mean annual rainfall is 871 mm.

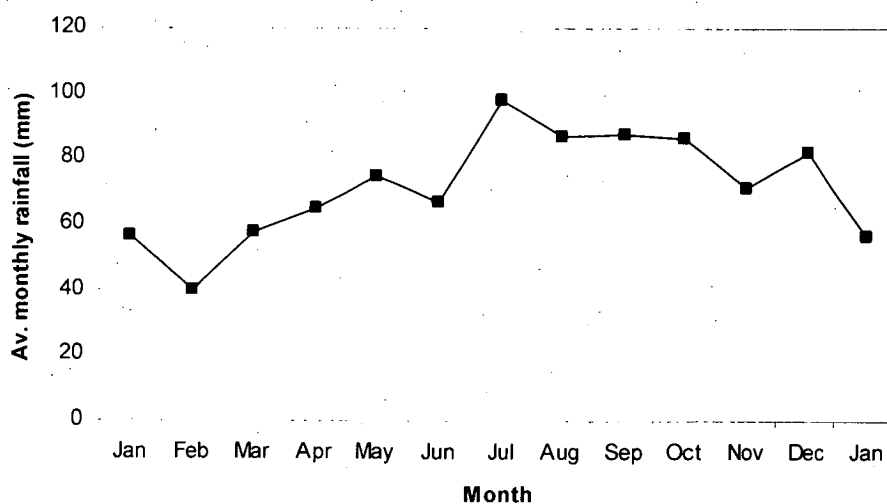


Figure 2.26 Long term (1972-1989) mean monthly rainfall for Fourfoot Road (Geeveston).

Table 2.4 Soil descriptions, drainage class and mean tree height at five sites along the transect study area at Geeveston in March 2003.

Plot (position)	Soil description	Drainage class	Mean tree height (m)
1 (0 m) Waterlogged plot	<u>A horizon</u> – Sandy loam 0-10 cm. <u>B1r horizon</u> – Grey clay to 10-70 cm. Gradual boundary. <u>B2g horizon</u> – Green and grey-mottled clay 70-90 cm. <u>BC horizon</u> – Orange clay 90-200 cm.	Very poorly drained	1.17
2 (50 m Tr plot 3)	<u>Ag horizon</u> – Grey clay to 0-15 cm. <u>B1g horizon</u> – Orange clay with grey mottling to a depth 15-50 cm. Gradual boundary <u>B2 horizon</u> – Orange clay 50-150 cm.	Poorly drained	1.46
3 (100 m Tr plot 5)	<u>A horizon</u> – Dark loam 0-15 cm. <u>B1g horizon</u> – Grey mottled grey clay 15-30 cm. Gradual boundary <u>B2 horizon</u> – Orange clay to 30-200 cm..	Imperfectly drained	2.95
4 (150 m) Drained plot	<u>A horizon</u> – Loam 0-10 cm. <u>B horizon</u> – Orange clay 10-150 cm.	Well drained	5.64

Soil descriptions

Inspection wells dug in February 2003 at 50 m intervals along established transects at both study areas determined that the waterlogged plots at both study areas met the classification of poorly or very poorly drained soil profiles (with greying to a depth of more than 0.6 m) (Table 2.4).

Growth

Tree height

At study areas 1 and 2, waterlogged trees were shorter than drained trees at the start of the study. By November 2004 drained trees had attained an average

height of > 7.5 m, with waterlogged trees only reaching a maximum height of 1.9 m (Figures 2.27 and 2.28). The seasonality of tree growth shown at Loloma was not evident at Geeveston. This could have been due to the sparsity of measurements at the Geeveston site, or to differences in climate and water availability.

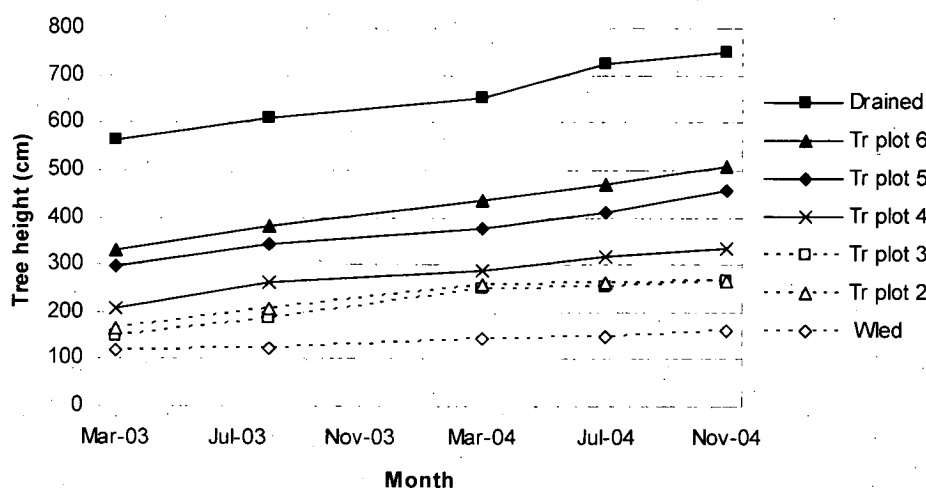


Figure 2.27 Average tree height (cm) for plots Tr 1 (waterlogged) to Tr 6 (drained) at study area 1 at Geeveston.

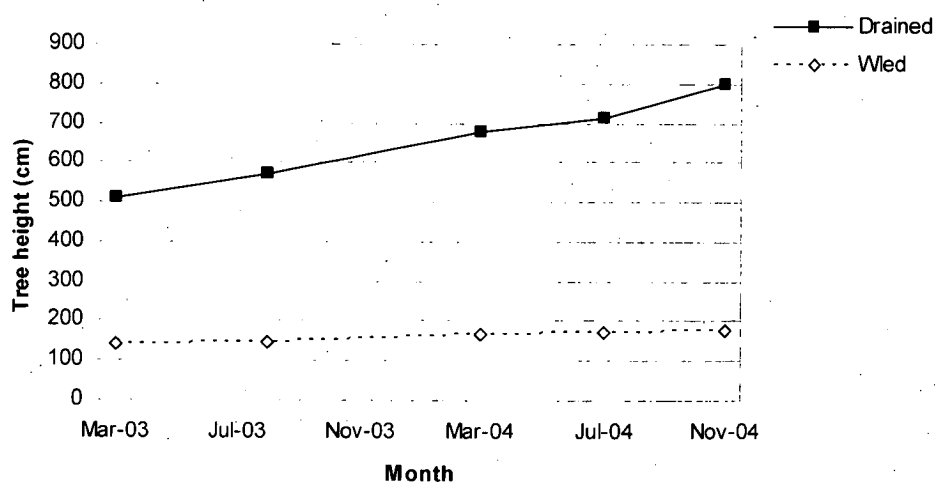


Figure 2.28 Average tree height (cm) of drained and waterlogged plots in study area 2 at Geeveston.

Tree volume

The average tree volume for each plot was calculated using the formula for conical volume in March 2003 and November 2004. There was a strong effect of waterlogging on tree stem conic volume. Waterlogged trees had a substantially smaller stem conic volume in comparison with drained trees, with the difference continuing to increase over the study period (Table 2.5). The volume of each plot at study site 1 increased with better drainage.

Table 2.5 Average tree volume (m^3) and standard error for study areas 1 and 2 at the southern field, calculated in March 2003 and November 2004.

Study plot	Position	Tree volume ($\text{m}^3 \times 10^{-3}$) March 2003	Tree volume ($\text{m}^3 \times 10^{-3}$) November 2004
<u>Study area 1</u>			
Tr plot 1 Waterlogged	0 m	0.47 ± 0.148	1.26 ± 0.58
Tr plot 2	25 m	2.17 ± 0.641	8.4 ± 2.0
Tr plot 3	50 m	1.70 ± 0.62	8.74 ± 2.6
Tr plot 4	75 m	6.11 ± 1.82	15 ± 4.8
Tr plot 5	100 m	6.63 ± 1.2	20.02 ± 9.6
Tr plot 6	125 m	12.6 ± 3.7	25.8 ± 4.5
Tr plot 7 Drained	150 m	47.7 ± 6.6	169 ± 25
<u>Study area 2</u>			
Waterlogged	0 m	0.81 ± 0.17	3.0 ± 0.57
Drained	150 m	61.4 ± 6.25	191 ± 29.3

2.4 Discussion

The selection of suitable eucalypt plantation sites in Tasmania is generally restricted to altitudes of above 300 m with rainfall greater than 1000 mm year⁻¹ (Orme et al. 1992, Honeysett et al. 1996). At higher altitudes low mean annual temperatures and high incidence of frost are strong environmental constraints on tree growth in Tasmania. The compounding effect of frost and waterlogging in the field is common due to the co-occurrence of these two environmental stresses in topographical depressions in the landscape (Davidson and Reid 1987). At Loloma the incidence of frost events in spring was found not to correlate with poor tree growth associated with waterlogged sites, therefore, frost was determined not to be a confounding environmental constraint on tree performance.

Waterlogged soil conditions can develop through fluctuations in perched or ground water tables. The presence of impeding soil layers, such as sharp boundaries in soil texture may result in a perched water table (McLaren and Cameron 1996). Soils with poor drainage due to the presence of perched water tables are identified by dominant grey colours overlaying yellow, brown or red layers (Laffan 2002). This typical evidence of a perched water table was recorded at both the Loloma and Geeveston field sites. At both field sites, the development of waterlogging occurred during June/July, at the time of peak winter rainfall and low evapotranspiration. This strong seasonal pattern of waterlogging facilitates the accurate prediction of the onset of waterlogging in the field, and may be useful in the management of waterlogged areas.

Two independent water tables were identified at Loloma. The deep water table was fed by an aquifer and rose later and fell earlier than the shallow water table. The shallow perched water table developed above the clay B horizon and was fed by run-on. Both water tables rose during June/July, with water remaining in shallow wells at waterlogged plots for up to five months (at Loloma study area 2), resulting in a saturated root zone for young trees. Therefore, current seasonal waterlogging may contribute to losses in tree production at Loloma.

The deep water tables at all drained plots at Loloma had receded to below 3' m by December, and did not reappear until the end of summer (March). In comparison, waterlogged plots had water within 1.5 m of the soil surface throughout the summer at two of the three areas. Due to the reliance of eucalypt species on stored ground water during summer (Nicolls et al. 1982, Worledge et al. 1998), this significant reduction in water table height at drained plots may induce drought stress during summer. The midday photosynthetic rate of drained trees was reduced during summer, which was probably due to poor access to soil water and the development of water stress under the conditions of high light and temperature. Coinciding with reduced photosynthetic rates, drained trees exhibited elevated lutein, β -carotene, and xanthophyll pigments during summer which is further indicative of drought stress. At waterlogged plots in summer, water was more readily available to tree root systems, with trees displaying no physiological stress.

The physiological response to waterlogging varies between tolerant and intolerant species. Intolerant species typically exhibit early stomatal closure (Kozlowski and Pallardy 1979, Beckman et al. 1992), and maintain reduced stomatal conductance throughout the waterlogging period. Waterlogging-tolerant species typically re-open stomata after a period of exposure (Tang and Kozlowski 1984, Pezeshki 1993). The development of physiological stress during exposure to waterlogging is characterised by reduced leaf water potentials, stomatal conductance, net photosynthesis and photosynthates utilisation (Chen et al. 2002, Huang et al. 1994, Vartepetian and Jackson 1997).

At Loloma the net photosynthetic rate of waterlogged trees was significantly reduced during July, coinciding with the development of waterlogging, but otherwise there was little evidence of physiological stress, with trees maintaining photosynthetic rates above $12-14 \mu\text{mol m}^{-2} \text{s}^{-1}$ (near maximum values of $15-20 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the species (Whitehead and Beadle 2003)) during spring and summer. Waterlogged trees had a greater availability of soil water during summer and maintained high rates of photosynthesis (up to maximum values for the species), corresponding to the relatively high growth rates observed from mid

summer to autumn. These results indicate the seasonal advantage of higher water tables at waterlogged plots.

The development of water stress (significantly lower predawn leaf water potentials) was not observed in the field during the waterlogging season. Eucalypts can exhibit water potentials of below -4 MPa under conditions of stress, therefore, the values recorded here are not extreme. Therefore, the observed reduction in photosynthetic rate of waterlogged trees is attributable to mechanisms other than water stress. The development of photoinhibition ($F_v/F_m < 0.65$) was recorded at one waterlogged plot during March 2003, which coincided with a reduction in foliar chlorophyll and xanthophyll concentrations. This result was not consistent with other waterlogged plots. At the same time in 2004, there was no indication of reduced F_v/F_m at any plot. There was also no indication of photoinhibition during the waterlogging period, though there was an increase in the xanthophyll conversion ratio throughout the waterlogging season (July – September).

The difference between the waterlogged and drained plots at Loloma, in terms of water table depths, became less significant as one moved from study area 1 to area 3. This was probably related to the relative position of the three areas in the landscape, and the movement of water through the study site. The slight decrease in elevation from area 1 to 3 (see map, Plate 2.2) and the close proximity of the drained plots to the waterlogged plots at areas 2 and 3, explains the progressive decrease in the difference in the water table fluctuations of drained and waterlogged plots from area 1 to 3.

The development of hypoxia in the soil solution at Loloma was observed in August, after 2 months of waterlogging. Dissolved oxygen (DO) concentration in deep wells at waterlogged plots were significantly lower than at drained plots across all study areas. Soil pH and redox potential were affected by the development of anaerobic conditions (Phillips 1999). However, there were no consistent changes in soil pH or redox potential between waterlogged and drained plots.

Soil greying is indicative of a permanently anaerobic soil environment as a result of long-term waterlogging (Fenwick and Knapp 1982). Prolonged anaerobic conditions result in the reduction of mineral elements, particularly iron and manganese (Hillil 1998). The chemical reduction of ferric ions (Fe^{3+}) present in iron oxides to the more soluble ferrous ion (Fe^{2+}) is partly responsible for the development of the grey colouring of waterlogged soils, in conjunction with the removal of ferric oxide coatings from other mineral particles, revealing their natural grey colours (McLaren and Cameron 1996). At both field sites, soil greying (predominantly grey or grey-mottled regions) was noted to a depth of greater than 0.6 m at all waterlogged plots, which is characteristic of long-term waterlogging, and poorly to very poorly drained soils (Laffan 2002). This typical greying of the soil profile was present at plots with seasonal inundation (during June to September) and poor tree productivity at both field sites. Therefore, soil profile investigations provide the most effective means of assessing the potential loss of productivity associated with waterlogging.

In association with changes in soil structure and nutrient availability, waterlogging has been shown to result in the loss of fine root biomass (Kozlowski 1979). This will have a severe effect on the growth and nutrition of trees during spring and summer (Bange et al. 2004) under conditions of rapid growth. Fine root biomass reaches quasi-steady state early in eucalypt stand development (Resh et al. 2003). If waterlogged trees have interrupted development characterised by the sequential loss and regrowth of fine roots, this will have a severe impact on tree allometric relationships and overall growth. Fine root growth represents a large proportion of net primary productivity (Janssens et al. 2002) and roots need to be replaced frequently to ensure optimal functioning of the root system. Therefore, during periods of high growth rates in spring and summer, waterlogged trees will have a substantially reduced size and functional capacity of the root system, which in conjunction with the reduced availability of essential elements, may explain the shift in the seasonality of growth and decreased growth rates of waterlogged trees in the field.

The intensity of waterlogging might be expected to decrease as a plantation grows. Evapotranspirational capacity increases rapidly during the early years of growth, and stabilises once canopy closure is attained (Worledge et al. 1998; Honeysett et al. 1996). Therefore, with increasing water use during the first 4-5 years of plantation growth, the incidence and severity of waterlogging is expected to decline. At the conclusion of the field study, waterlogged trees had a tree height of only 60 % of that of drained trees. At the Geeveston site, waterlogged trees had a mean height of only 22 % of that of drained trees after 7 years growth. The stem conic volume of trees reflected the results of the height data. Drained plots produced significantly higher tree volumes than waterlogged plots through the initial 3 years of plantation growth. There was an average of 75 % loss in mean conic volume of waterlogged trees after 3 years. These significant losses in tree productivity for *E. globulus* are consistent with those described for *E. nitens* (Connell 1998, Mummery et al. 1999). This result indicates there is a potentially serious effect of waterlogging on productivity of *E. globulus* plantations. Despite the reduction in the severity of waterlogged conditions over the life of the plantation, there is a cumulative effect of waterlogging on tree productivity, which increases with plantation age.

Relative growth rate (RGR) in height of all trees at Loloma was found to decrease with plantation age. The waterlogged trees had a lower RGR than drained trees in year 1, but higher RGR in subsequent years (2 and 3). However, the difference in tree height between drained and waterlogged trees continued to increase. This indicates that the damage incurred during the first waterlogging season has a long term effect on plantation productivity. Therefore, if waterlogging damage can be minimised and/or ameliorated during the first year of plantation growth, the overall loss in productivity due to waterlogging may be significantly reduced. This result also indicates that only one year of specialised and intensive management, which may include options such as the application of fertiliser, changes in planting times or additional mounding in order to minimise the effects of waterlogging.

~ CHAPTER 3 ~

The effect of long term waterlogging on the growth and physiology of *E. globulus* and *E. nitens***3.1 Introduction**

Waterlogging is widespread across the hardwood plantation estate in Tasmania. The severe effect that waterlogging can have on eucalypt productivity in the field was demonstrated at both Tasmanian field sites (Loloma and Geeveston, Chapter 2). This study also demonstrated that it is the first year's growth that is severely affected by waterlogging. Therefore, it is important to understand the physiological response of eucalypt seedlings to waterlogging during the first year. Controlled environments within a glasshouse provide a practical means of investigating early growth, stress physiology and morphological responses to waterlogging. This chapter investigates the first year of growth of the major plantation species, *E. globulus* and *E. nitens*, in response to waterlogging.

The response to waterlogging is dependent on plant age, the timing and duration of waterlogging and chemical properties of the medium (Nicolas et al.. 2005, Yordanova 2005). Waterlogging early in vegetative growth or during periods of rapid growth, is more damaging than exposure to waterlogging during late vegetative or reproductive phases (Wetson et al.. 1976, Angelov et al.. 1996).

After prolonged exposure to waterlogging, there may be extensive root death that occurs in response to the development of hypoxia in the root zone, and reduced availability of respiratory substrates. The degree of reduction in photosynthetic rate and pool of respiratory substrates is dependent on the duration of exposure to hypoxic conditions (Yordanova et al. 2005). Even short term or transient waterlogging events can have significant effects on plant growth and productivity (Jackson 1979, Sharma and Swarup 1988, Melhuish et al.. 1991). In wheat, exposure to waterlogging periods of only one to two days has been reported to significantly decrease final yield (Melhuish et al.. 1991).

A reduction in photosynthetic performance under waterlogged conditions may be associated with the development of a nutrient imbalance or deficiency. The development of foliar nutrient deficiency in waterlogged plants has been reported in a number of species, including eucalypts (Hook et al. 1983, Van der Moezel et al. 1988, Bacanamowo and Purcell 1999, Malik et al. 2001, 2002, Close and Davidson 2003). Foliar deficiency in phosphorus has been shown to reduce the rate of photosynthesis (Plesnicar et al. 1994, Kirschbaum and Tompkins 1990). There is also evidence of nitrogen deficiency reducing the maximal efficiency and quantum yield of Photosystem II (PSII) (Verhoeven et al. 1997), stomatal conductance (Huang et al. 2004) and increasing xanthophyll cycle engagement (Lu et al. 2001, Cheng 2003) indicating the development of photoinhibition. The development of photoinhibition has been reported in a number of species under waterlogged conditions, including *E. nitens* (Close and Davidson 2003).

The most significant morphological adaptation to waterlogging is the development of aerenchyma in the stem and root which acts as an internal air-conducting pathway (Kawase and Whitmoyer 1980). The development of adventitious roots, stem hypertrophy and the production of aerenchyma are morphological adaptations which have been correlated with waterlogging tolerance in *E. viminalis*, *E. ovata*, *E. robusta*, *E. camaldulensis* and *E. globulus* (Clemens and Pearson 1977, Ladiges and Kelso 1977, Sena Gomes and Kozlowski 1980, Blake and Reid 1981).

Connell (1998) reported that differences in the growth rates of *E. nitens*, *E. regnans* and *E. globulus* were due, in part, to differences in the ability of the species' for morphological adaptation to waterlogging. The capacity to develop basal stem hypertrophy and adventitious roots was only seen in the two Symphyomyrtus species, *E. globulus* and *E. nitens* (Connell 1998). This suggests that the capacity of *E. globulus* and *E. nitens* to survive and tolerate waterlogging is related to their ability to develop morphological adaptations. Significant reductions in the height growth of waterlogged *E. globulus* and *E. nitens*, of up to 73% have been reported (Connell 1998), which is consistent with that described

by Marcar et al. (2002) of a 70 % reduction in the shoot dry weight of *E. globulus* under waterlogged conditions.

Typical physiological responses of plants to waterlogging include reduced photosynthetic rate, reduced transpiration and stomatal closure (Reece and Riha 1991, Pezeshki 1993). The rapid reduction in photosynthesis has been attributed to stomatal closure (Kozłowski and Pallardy 1979, Sena Gomes and Kozłowski 1986), and has been associated with a decrease in root hydraulic conductivity under waterlogged conditions (Andersen et al. 1984, Davies and Flore 1986, Reece and Riha 1991). However, several other studies, including work on a number of eucalypt species, have reported that stomatal closure was not induced by leaf water stress (Periera and Kozłowski 1977, Tang and Kozłowski 1984, Zang and Davies 1986) indicating that other mechanisms are responsible for these early physiological responses.

The aim of this study was to quantify the physiological, morphological and growth responses of *E. globulus* and *E. nitens* seedlings to long-term waterlogging during the first year of growth.

Hypotheses tested;

1. There is a difference between *E. globulus* and *E. nitens* seedlings in the capacity for anatomical and morphological adaptation to waterlogging.
2. Soil type affects the growth and physiology of waterlogged *E. globulus* and *E. nitens* seedlings.
3. There is a difference in the physiological response of *E. globulus* and *E. nitens* to long-term waterlogging.
4. Plant nutrient status plays a role in the development of waterlogging injury through the development of photoinhibition.

3.2 Materials and methods

3.2.1 Experimental design

Seedling stock

Two hundred and fifty, 20 cm tall *Eucalyptus globulus* and *Eucalyptus nitens* seedlings were supplied by Woodlea Nursery Scottsdale, Tasmania, in November 2002. *Eucalyptus globulus* seed was collected from the Boral seed orchard at Winkleigh in 2000. *Eucalyptus nitens* seed was sourced from the Bream Creek orchard (Derford provenance).

Soil collection

Two native soil types were collected from a site at Geeveston, southern Tasmania in November 2002. A sandy loam A-horizon from a soil profile formed on mudstone bedrock and a clay loam A-horizon formed over a dolerite bedrock were collected. The soil was passed through a 3 cm sieve to remove larger fragments of wood and rock. Both soil types were thoroughly mixed and kept moist until planting.

Potting conditions

Seedlings of both species were individually potted into 4.5 L plastic pots 20 cm in diameter. Coarse gravel was placed to a depth of 5 cm in the bottom of pots to facilitate drainage. Pots were filled to within 5 cm of the top with either dolerite or mudstone soil. Seedlings were well watered immediately after planting and daily thereafter. All seedlings were grown for 5 months under glasshouse conditions before treatments were imposed.

Treatments

1. Species; *E. globulus* and *E. nitens*.
2. Soil types; topsoil developed over a dolerite or mudstone bedrock.
3. Fertilisation; Fertiliser was applied to half the pots for the first 4 months of the establishment period as a three times weekly application of 200 mL of Peters Excel®, at a concentration of 1 g L^{-1} . The other half of the pots remained non-fertilised throughout the experiment.

4. Waterlogging; after a 5 month establishment period, half of the fertilised and half of the non-fertilised pots were randomly allocated to the waterlogging treatment. Waterlogged conditions were applied by submerging pots in 10 L plastic buckets filled with tap water. The water level was maintained at the soil surface for the 28 weeks. Control pots remained freely drained and were watered daily throughout the experimental period.

Design;

= 2 species x 2 soil types x 2 fertiliser treatments x 2 waterlogging treatments
= 16 treatments

This design resulted in a total of 16 treatment groups, each treatment had 30 replicates. Treatments were organised in a randomised block design and grown under glasshouse conditions from December 2002 – November 2003. (Mean day and night summer temperature; 24°C and 17.5°C. Mean day and night winter temperatures; 17.4°C and 10.5°C).

3.2.2 Measurements

Growth and survival

Seedling survival and height was measured monthly on 10 seedlings from each treatment, from December 2002 until the completion of the experiment in November 2003.

Relative growth rate (RGR) of seedling height and biomass were calculated. The formula for relative growth in height was;

$$\text{RGR} = \text{Ln}(\text{Height}_2) - \text{Ln}(\text{Height}_1) / \text{Time}_2 - \text{Time}_1$$

Biomass harvests of three randomly selected seedlings per treatment were conducted at the following times; (1) prior to the imposition of waterlogging in April 2003, (2) after 20 weeks of waterlogging in September 2003, and (3) at the completion of the experiment, after 28 weeks of waterlogging in November 2003. At each harvest, plant height, stem diameter, stem stele diameter (at soil surface), leaf number and the fresh weight of root, stem and leaf were recorded. The

percentage stem cortex was calculated by subtracting the area of stele from the total stem cross-sectional area. Biomass samples were dried at 70 °C to constant weight to obtain dry weight.

Morphology

Root

Root systems were assessed at each harvest. Aerenchymatous roots were identified by their spongy white appearance and separated from the fibrous root tissue. The fresh weight of aerenchymatous and fibrous root tissue was recorded to calculate percentage (by weight) of the root system composed of aerenchymatous roots. The total number and length of the longest aerenchymatous root were recorded. The absence or presence of aerenchymatous root tissue in the primary root system was recorded.

Leaf

Qualitative observation of leaf morphology, including the development of necrosis and chlorosis, malformation, changes in leaf pigmentation and wax development was conducted.

Physiology

Physiology was studied on a subset of seedlings from the dolerite soil treatment only due to time limitations. Three replicate seedlings from each of the eight treatments applied to dolerite soils were randomly selected prior to each measurement. A total of 24 pots were measured. Measurements were conducted prior to waterlogging and after 3, 6, 10, 14, 17, 20, 24 and 28 weeks of waterlogging.

Leaf water potential

Predawn leaf water potential was measured at monthly intervals, using a pressure chamber (PMS Instrument Co., Corvallis, Oregon) (Scholander et al. 1965).

Mature leaves from the lowest third of the crown were excised and enclosed in the chamber and the balance pressure obtained within one minute.

Photosynthesis

Midday measurements (between 11.30am and 1.00pm) of net photosynthetic rate (photosynthesis – respiration) and stomatal conductance were conducted at a light intensity of 1000 μE , flow rate of 500 mL min^{-1} , and sample CO_2 concentration of 370 ppm. Recently fully expanded leaves of the upper third of the crown were selected for measurement using a portable photosynthesis system (LI-COR Li-6400).

Photochemical efficiency

Predawn F_v/F_m measurements were conducted on three recently fully expanded leaves using a portable PAM-2000 fluorometer (Heinz Walz GmbH, Elfelrich, Germany) and 2030-B leaf-clip holder. Leaves were immediately excised and frozen in liquid nitrogen and stored in a freezer at -20°C for future pigment analysis.

Pigment analysis

Analyses of chlorophyll and xanthophyll pigment concentrations were conducted using the acetone method, adapted from Close et al. (2003). See Chapter 2 for methodology.

Nutrient analysis

The youngest, fully expanded leaves were used for nutrient analysis and collected from the final harvest of seedlings. These leaves were selected as they represent the age of leaves with the highest nutritional status. Samples were ground and dried overnight at 70°C . Analysis of nitrogen, phosphorus, potassium, magnesium and calcium was conducted on *E. globulus* and *E. nitens* samples. Macronutrient analysis was conducted using the sulphuric acid/hydrogen peroxide digest method (Lowther 1980). Three blanks and three reference standards were included in each analysis. The reference standards used for quantification were Euc123, Pine 43 and Pine 9 and it was ensured that the results of these reference standards were within 5% of the known quantity of analyte.

Soil chemistry

PVC inspection wells were installed in a subset (three per treatment) of experimental pots to facilitate the ongoing monitoring of soil solution pH, dissolved oxygen (DO) and redox potential (Eh). Measurements were made on days 1, 2, 4, 5, 6, 7, 14, 21 and 49, using the handheld dissolved oxygen – pH – mV – temperature meter; model WP-91 (TPS Pty Ltd.).

3.2.3 Statistical analysis

A four-way analysis of variance was performed using the proc and GLM procedures of SAS (SAS Institute, 1996) to analyse the effects of waterlogging, fertilisation, soil type and species on growth measurements at the three harvests. Physiological measurements were analysed similarly. All variables were checked for normality and transformed, using log transformations if required.

3.3 Results

3.3.1 Soil structure

The structure of the mudstone soil was altered in the process of collection, sieving, potting and waterlogging. It was observed to change from a loosely-packed clay-loam to be more dense and compacted under waterlogged conditions. The dolerite soil did not show the same alteration in structure under waterlogged conditions.

3.3.2 Soil chemistry

pH

The pH of waterlogged soil declined rapidly, from pH 7 to approximately pH 5 in both soil types over the first seven days of waterlogging (Figure 3.1). The pH had regained near initial values after 14 days, after which it remained stable at pH 6.5 (in dolerite) over the measurement period of 49 days. There was one pH unit difference between the two soil types at the conclusion of measurements. Fertilisation had little effect in dolerite soil throughout the waterlogging period. In mudstone, fertilisation lowered soil pH by approximately 0.4 pH units after two weeks of waterlogging.

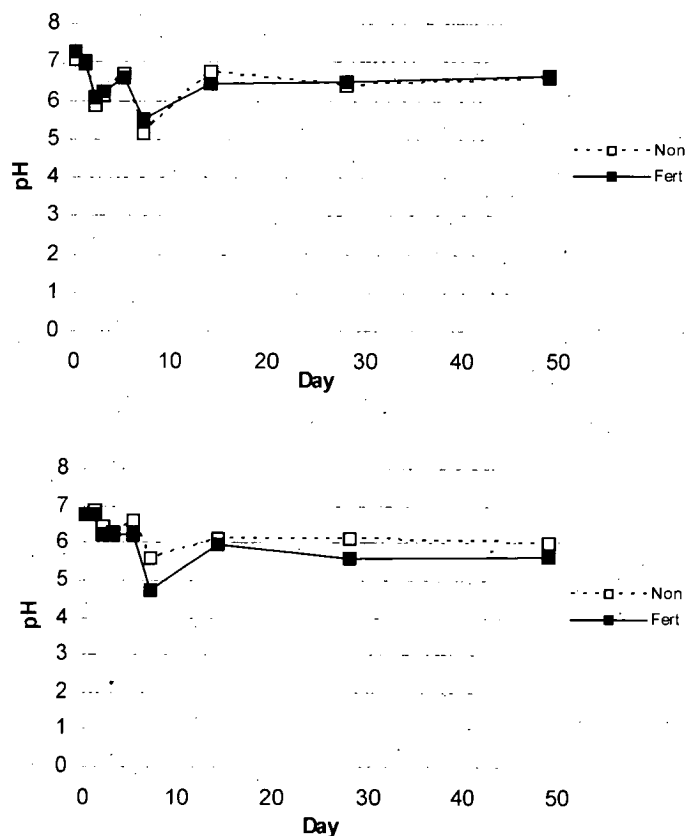


Figure 3.1 Soil solution pH in non-fertilised (Non) and fertilised (Fert) dolerite (above) and mudstone (below) soils over the initial 49 days of waterlogging.

Dissolved oxygen (DO) in the soil solution was similar for both soil types at the beginning of measurements (approximately 7 ppm) (Figure 3.2). Under waterlogged conditions DO declined steadily, reaching near zero levels in mudstone after 49 days. Mudstone soils had a lower final oxygen concentration and lower DO under fertilised than non-fertilised conditions. There was no significant effect of fertiliser on dolerite soil DO.

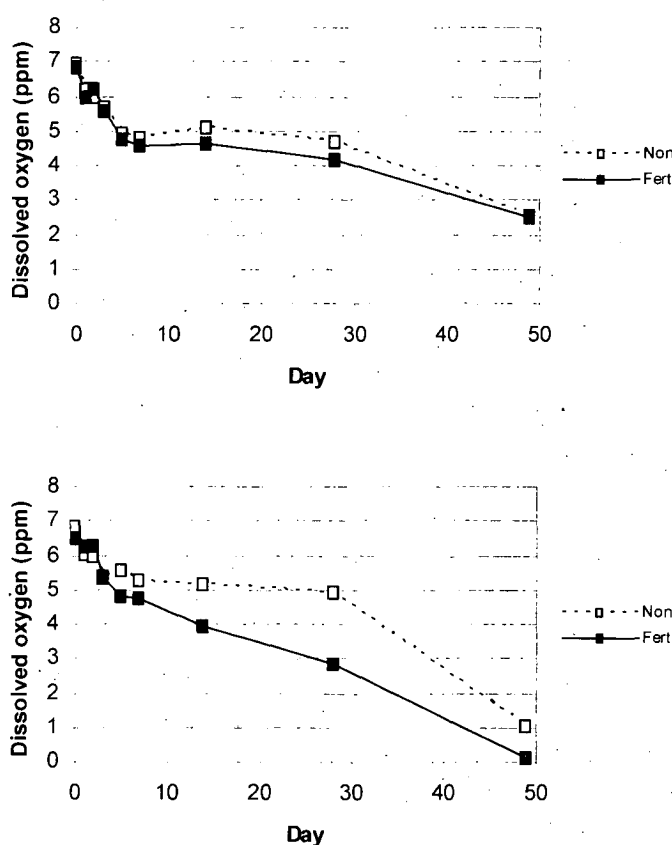


Figure 3.2 Soil solution DO in non-fertilised (Non) and fertilised (Fert) dolerite (above) and mudstone (below) soils over the initial 49 days of waterlogging.

Redox potential (Eh)

Initial soil solution redox potentials were similar for the two soil types (approximately 170 mV) (Figure 3.3). Dolerite exhibited a more rapid decline in Eh (to 30 mV) than mudstone (to 90 mV) over the 49 days of measurement. There was no significant effect of fertiliser on soil redox potential.

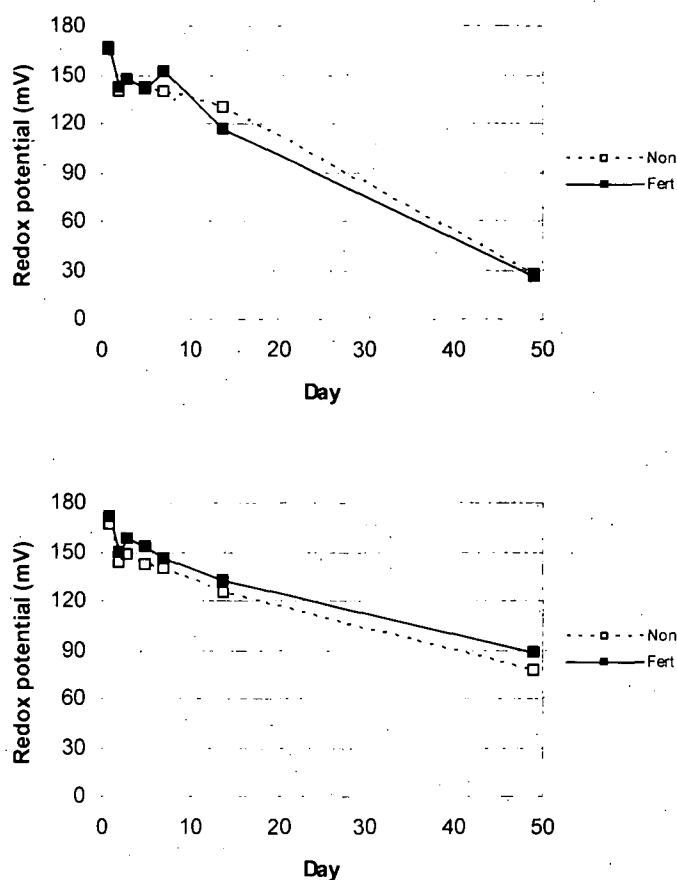


Figure 3.3 Soil solution redox potential (mV) in non-fertilised (Non) and fertilised (Fert) dolerite (above) and mudstone (below) soils over the initial 49 days of waterlogging.

3.3.3 Seedling morphology

Observed morphological changes induced by waterlogging included; stem hypertrophy (Plate 3.1), leaf loss, leaf spot and marginal necrosis (Plate 3.2), leaf chlorosis, death of apical buds, malformation of developing leaves, changes to leaf pigmentation (Plate 3.3), increased wax development on new growth (Plate 3.4), reduction in leaf area, shortened internode length and coppicing.



Plate 3.1 Stem hypertrophy in waterlogged/fertilised *E. globulus* seedling after 20 weeks of waterlogging.



Plate 3.2 Example of the development of leaf necrosis in a waterlogged *E. globulus* seedling.



Plate 3.3 Example of changes to leaf pigmentation and the development of anthocyanins in waterlogged *E. globulus* seedling.



Plate 3.4 Example of increased wax development in waterlogged *E. globulus* seedling. Note leaf curling.

3.3.4 Growth

Seedling height

From day -50 onwards, growth was higher in fertilised seedlings (Figure 3.4).

Following the imposition of waterlogging on day 0, fertilised and waterlogged *E. globulus* seedlings growing in dolerite had the same growth rate as fertilised and

drained seedlings, until day 100 of waterlogging, after which the growth rate of fertilised and waterlogged seedlings decreased, relative to fertilised and drained seedlings (Figure 3.4). A similar growth pattern occurred under non-fertilised conditions irrespective of waterlogging treatment (Figure 3.4). In mudstone, the growth of fertilised and waterlogged seedlings decreased immediately after imposition of waterlogged conditions and by the end of the experiment the height of drained fertilised and waterlogged fertilised seedlings differed greatly. The same trend was evident for drained non-fertilised and waterlogged non-fertilised treatments. *E. globulus* had a higher mean final height in dolerite than mudstone across all treatments, except the fertilised and drained (Figure 3.4).

Eucalyptus nitens had a similar growth pattern to *E. globulus* in response to treatments. For drained/fertilised seedlings, in both soil types, *E. nitens* attained an almost identical height to *E. globulus*. However, *E. nitens* grew more slowly under waterlogged/fertilised conditions in both soil types and more rapidly under the drained/non-fertilised treatment in dolerite than *E. globulus*.

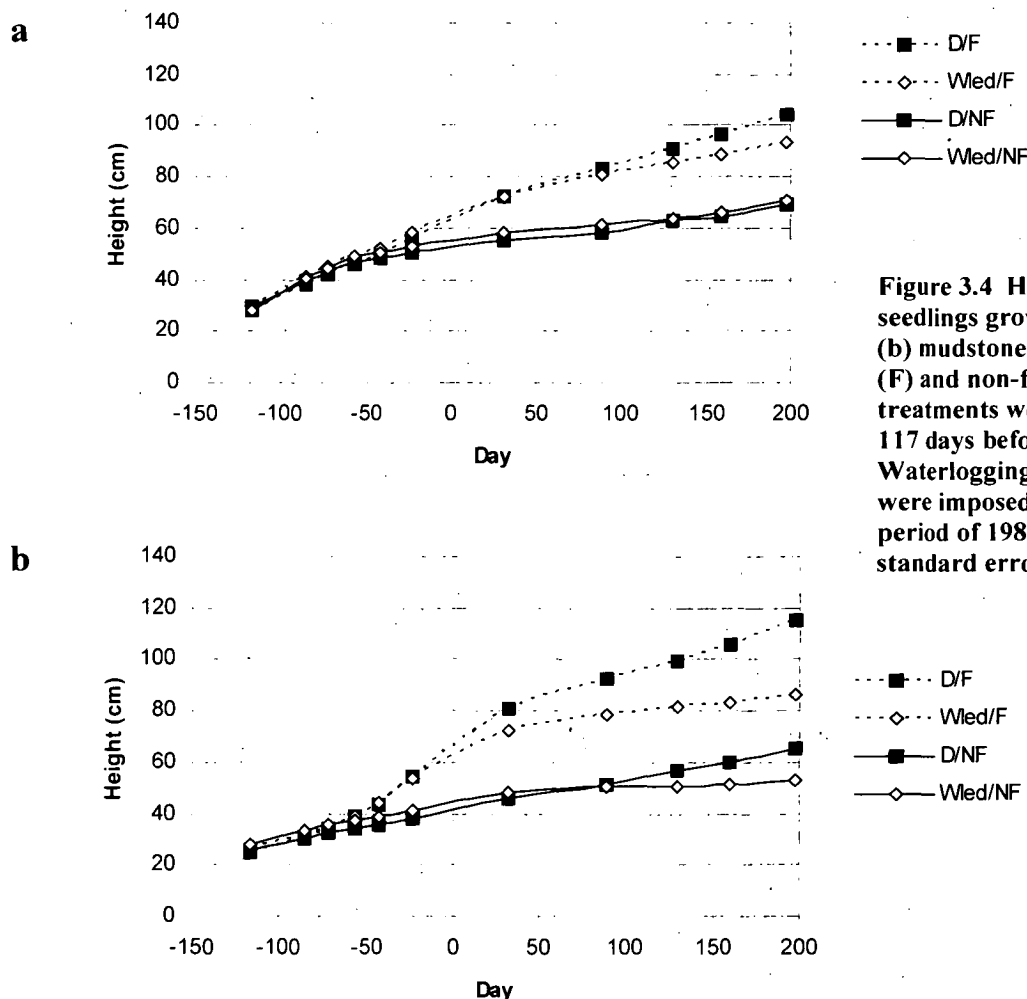


Figure 3.4 Height of *E. globulus* seedlings grown in (a) dolerite and (b) mudstone field soils. Fertilised (F) and non-fertilised (NF) treatments were maintained for 117 days before waterlogging. Waterlogging treatments (Wled) were imposed at day 0 for a period of 198 days. Largest standard error = 6.6 cm.

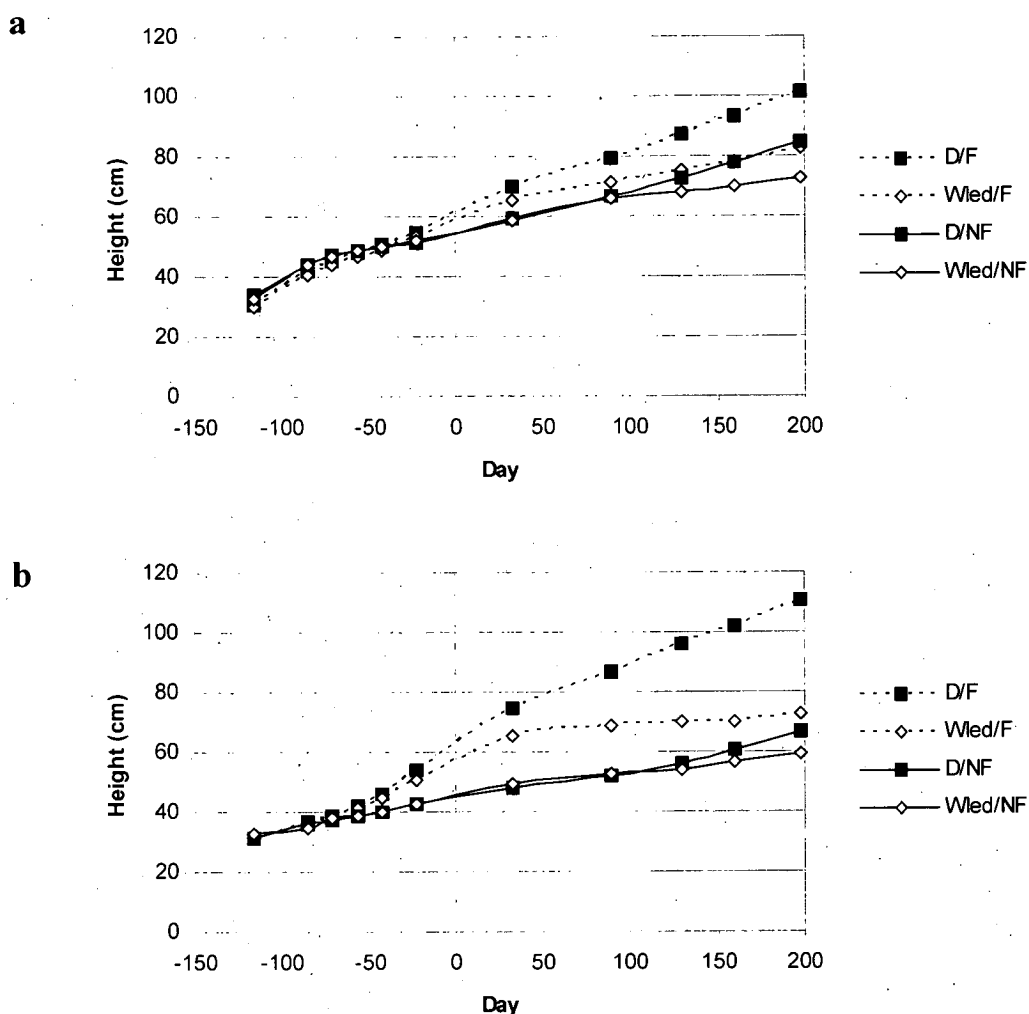


Figure 3.5 Height of *E. nitens* seedlings grown in (a) dolerite and (b) mudstone field soils. Fertilised (F) and non-fertilised (NF) treatments were maintained for 117 days before waterlogging. Waterlogging (Wled) and drained (D) treatments were imposed at day 0 for a period of 198 days. Largest standard error = 7.5 cm.

Relative growth rate (RGR) in height

There was a general decline in seedling RGR as the experiment grew into winter (Figures 3.6 and 3.7). Fertilisation during the first four months resulted in higher RGR in both species and soil types. At the end of the 28 week waterlogging treatment (day 198) all seedlings in dolerite had a similar RGR. In the mudstone soil, drained treatments of both species had a higher RGR than waterlogged treatments.

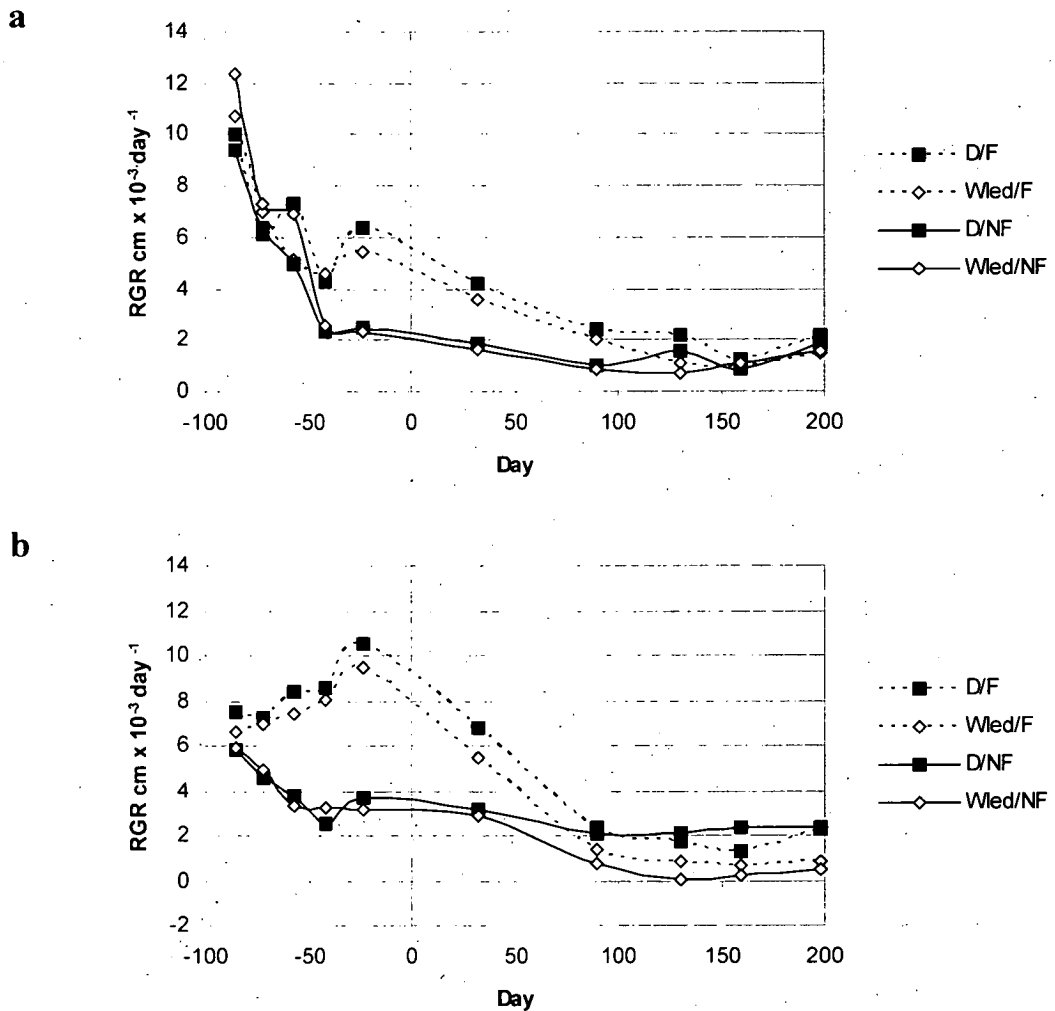


Figure 3.6 Relative growth rate (RGR) in height of *E. globulus* in (a) dolerite and (b) mudstone. The onset of establishment growth was at 117 days prior to the onset of waterlogging at Day 0. Waterlogging was imposed for a period of 198 days.

Largest standard error = 0.067 cm.

Wled = waterlogged, D = drained, NF = non-fertilised, F = fertilised.

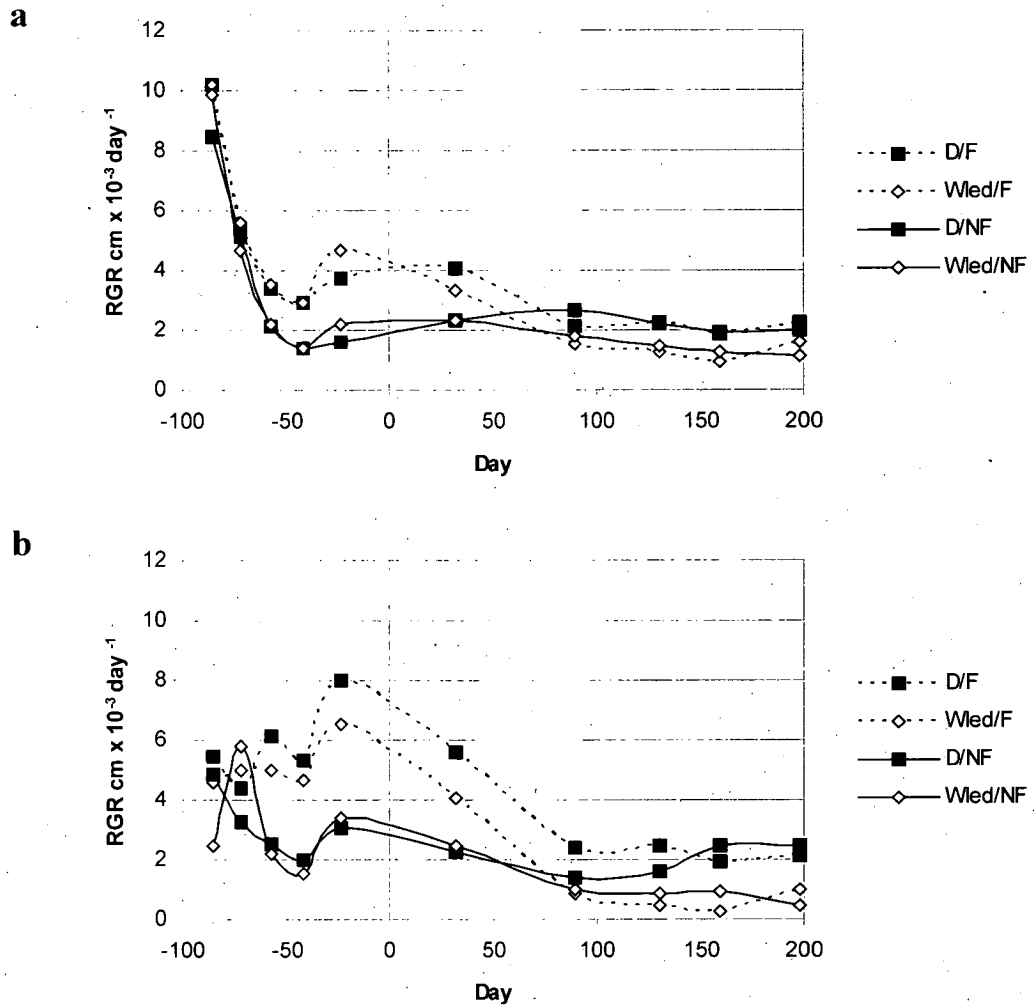


Figure 3.7 Relative growth rate (RGR) in height of *E. nitens* in (a) dolerite and (b) mudstone. The onset of establishment growth was at 117 days prior to the onset of waterlogging at Day 0. Waterlogging was imposed for a period of 198 days.

Largest standard error = 0.079 cm.

Wled = waterlogged, D = drained, Non = non-fertilised, Fert = fertilised.

Biomass

Prior to waterlogging at harvest 1 (day -1), *E. nitens* had a significantly higher biomass than *E. globulus* in all treatments ($P < 0.0001$). Unfertilised dolerite substrate was more productive than unfertilised mudstone. Fertiliser application removed the soil effect. There was no effect of fertiliser application on plant biomass in the dolerite soil (Figure 3.8).

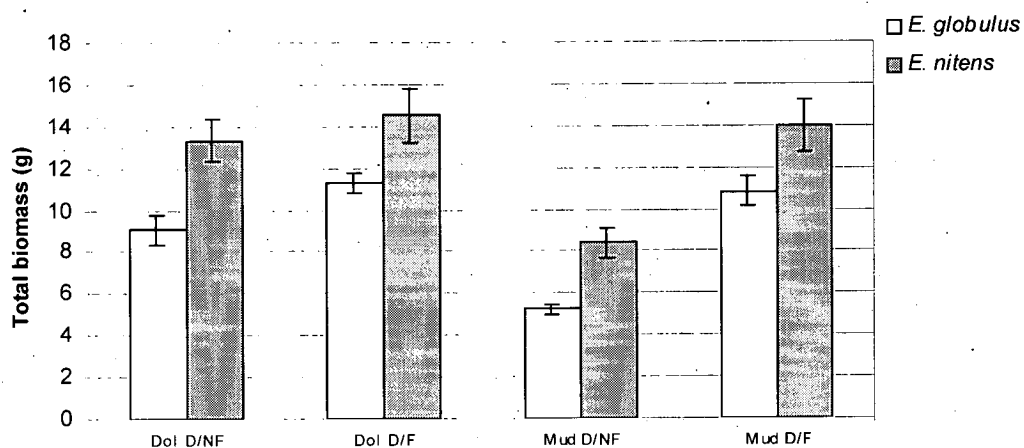


Figure 3.8 Total seedling dry weight (g) of *E. globulus* and *E. nitens* at harvest 1 (day -1) in dolerite (Dol) and mudstone (Mud) soil types. Drained = D, fertilised = F and non-fertilised = NF. Bars represent standard error.

There was a significant effect of species, soil type and fertiliser application (all $P = 0.0001$, Table 3.1) on seedling stem diameter prior to waterlogging. *Eucalyptus nitens* had a higher mean stem diameter than *E. globulus* (Figure 3.9). Seedlings in dolerite had a higher mean stem diameter than those in mudstone across all treatments and species (Figure 3.9).

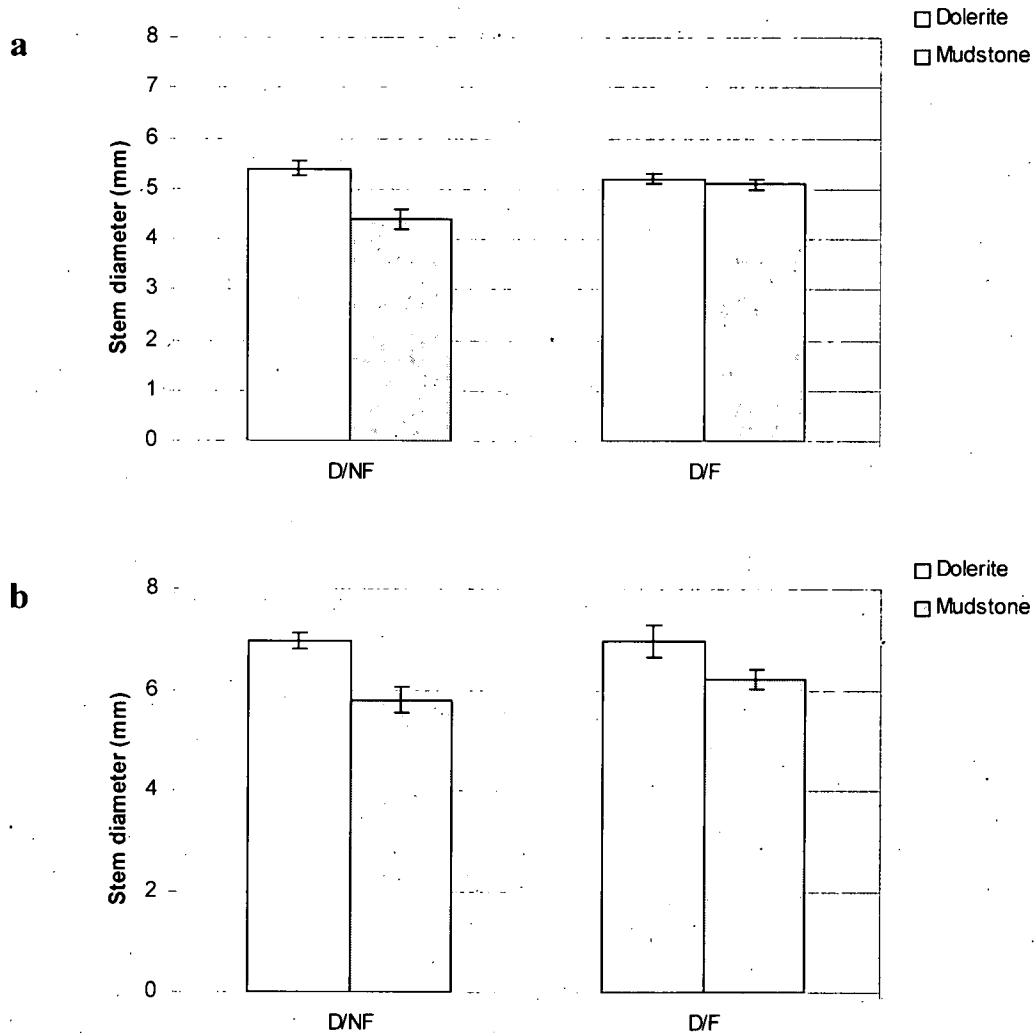


Figure 3.9 Mean stem diameter of (a) *E. globulus* and (b) *E. nitens* seedlings prior to the onset of waterlogging at harvest 1 in dolerite and mudstone soil types. Drained = D, fertilised = F and non-fertilised = NF. Bars represent standard error.

Prior to waterlogging, *E. globulus* seedlings had a higher root:shoot ratios in dolerite than mudstone substrate (Figure 3.10). The root:shoot ratio of *E. nitens* was lower in fertilised treatments in both soil types (Figure 3.10): *Eucalyptus nitens* seedlings had higher root:shoot ratios in dolerite soil under fertilised conditions, and in mudstone soil under non-fertilised treatments (Figure 3.10).

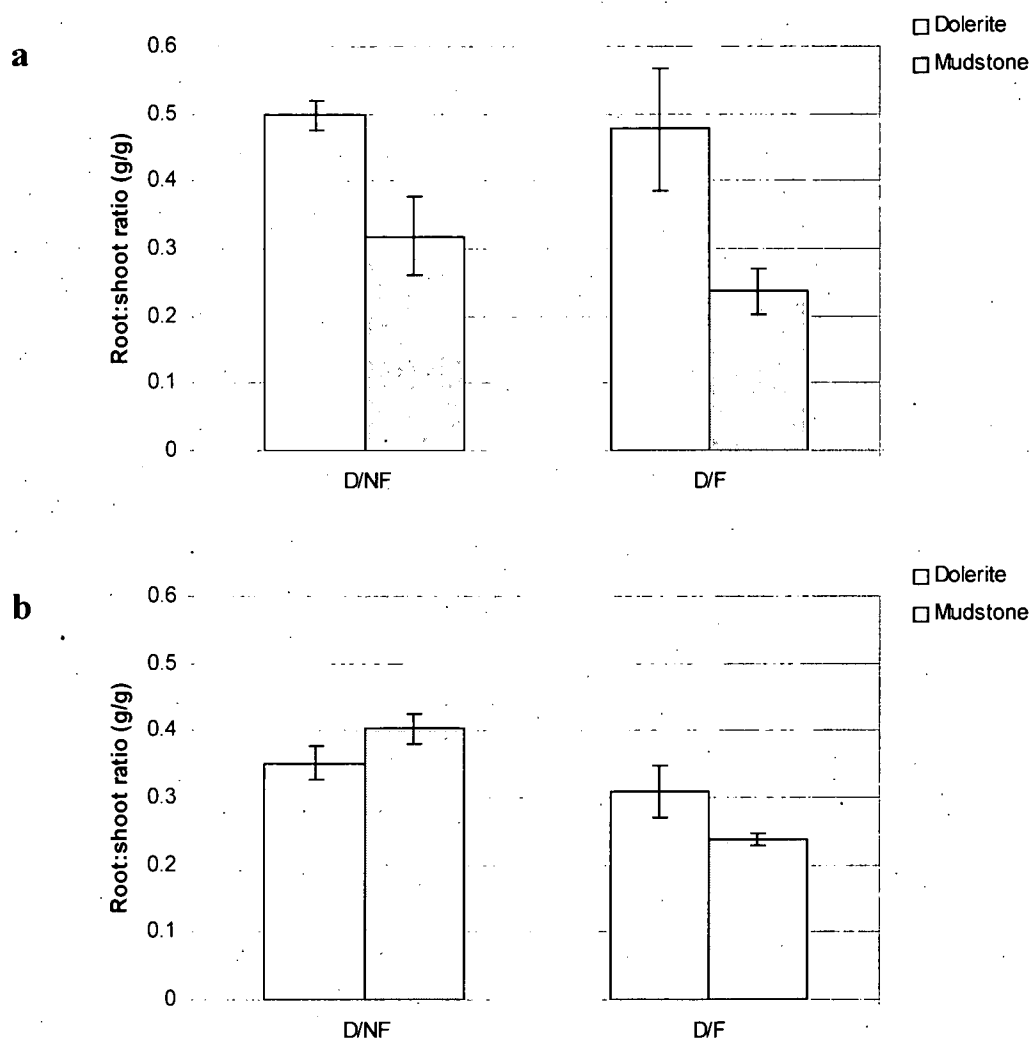


Figure 3.10 Mean root:shoot ratio of (a) *E. globulus* and (b) *E. nitens* seedlings prior to the onset of waterlogging at harvest 1 in dolerite and mudstone soil types. Drained = D, fertilised = F and non-fertilised = NF. Bars represent standard error.

After 20 weeks of waterlogging (harvest 2) a shift in the pattern of biomass accumulation was observed (Figure 3.11). There was a significant increase in

plant biomass in both species after fertilisation in both soil types under drained and waterlogged conditions ($P < 0.0001$), except for *E. nitens* in drained and fertilised dolerite. *Eucalyptus globulus* had a higher growth rate in all fertilised treatments and *E. nitens* had higher growth in all non-fertilised treatments.

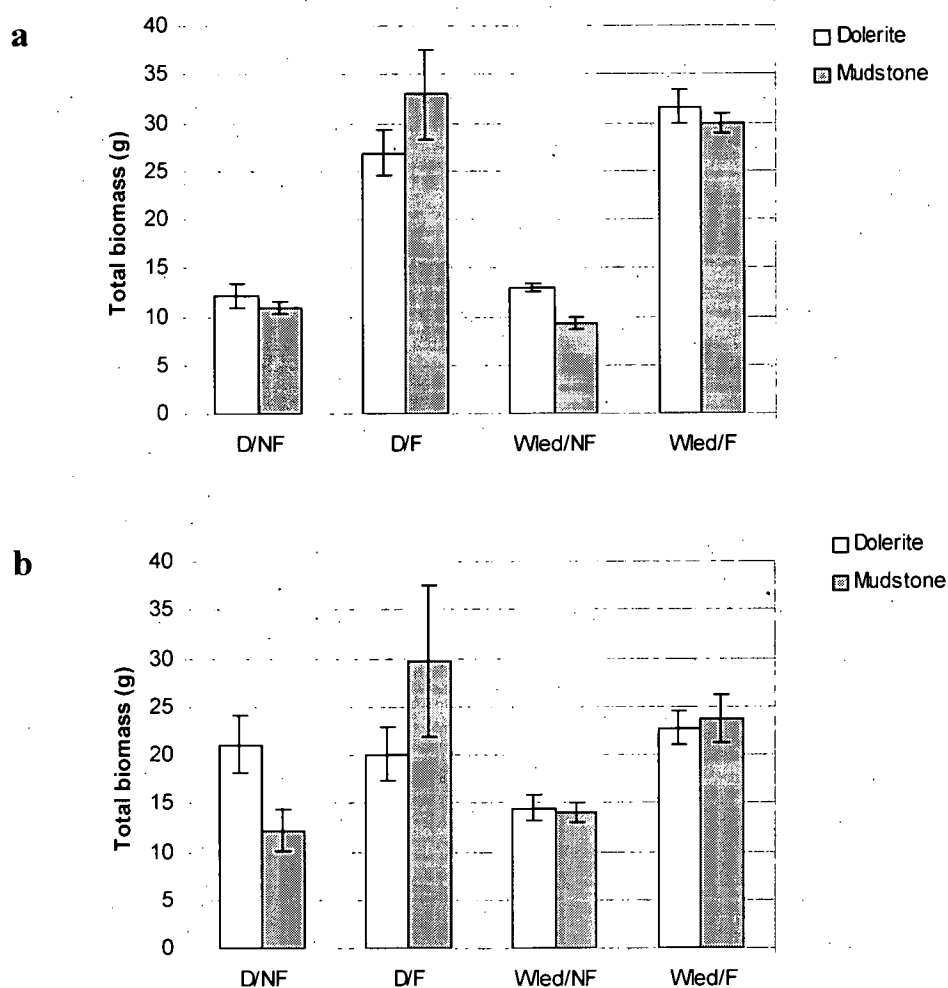


Figure 3.11 Total seedling dry weight (g) of (a) *E. globulus* and (b) *E. nitens* at harvest 2 (after 20 weeks of waterlogging) in dolerite (Dol) and mudstone (Mud) soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

At harvest 2, after 20 weeks of waterlogging, there were significant effects of waterlogging ($P = 0.0001$) and fertilisation ($P = 0.0001$) on seedling stem diameter. In *E. globulus*, stem diameter was greatest under a combination of fertilised and waterlogged conditions, and least under a combination of drained and non-fertilised treatments (Figure 3.12a). This trend was less evident in *E. nitens* (Figure 3.12b).

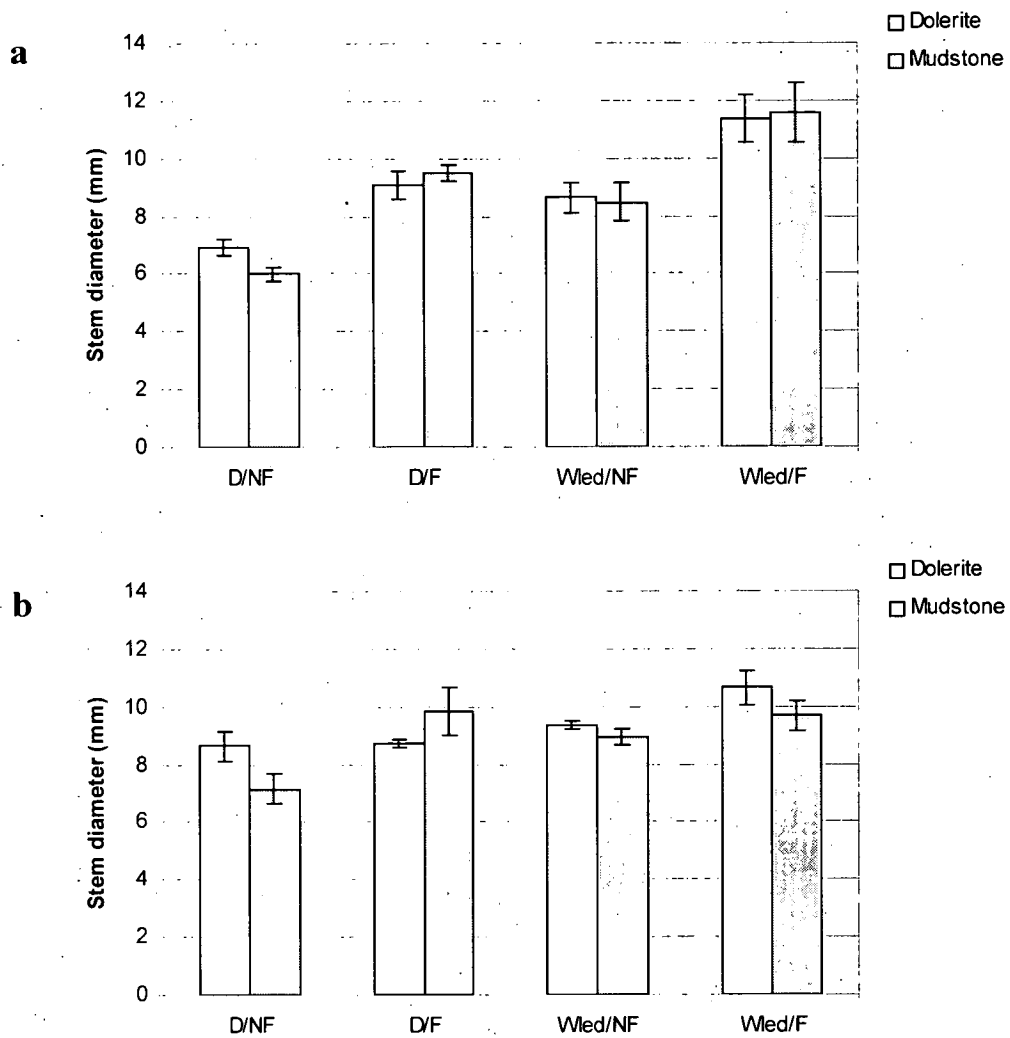


Figure 3.12 Mean stem diameter of (a) *E. globulus* and (b) *E. nitens* at harvest 2 (after 20 weeks of waterlogging) in dolerite and mudstone soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

The percentage of stem cortex after 20 weeks of waterlogging was significantly different between species ($P = 0.0024$) and in response to waterlogging ($P = 0.0001$). *Eucalyptus globulus* had a higher percentage of stem cortex than *E. nitens* (Figure 3.13). There was a significant increase in the development of stem hypertrophy (an increase in stem cortex) under waterlogged conditions in both species and for both soil types (Figure 3.13).

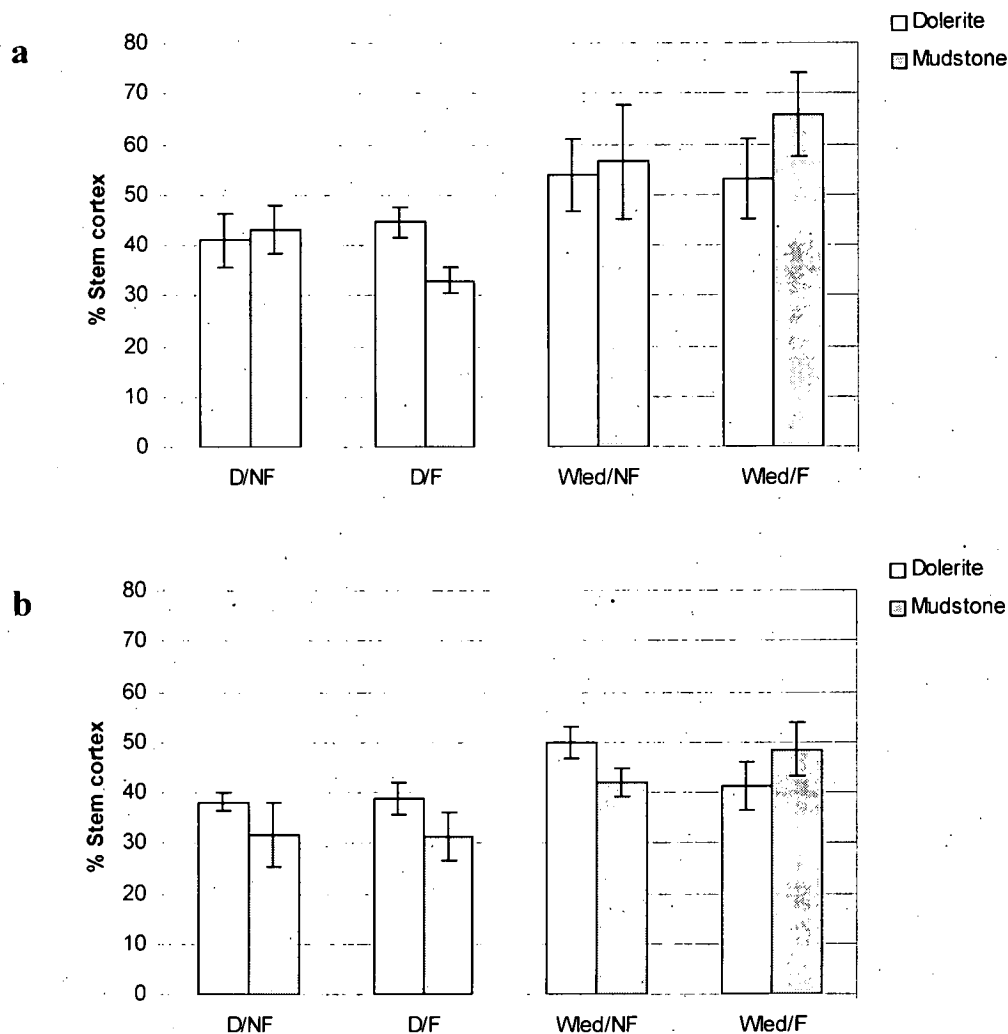


Figure 3.13 Percentage stem cortex of (a) *E. globulus* and (b) *E. nitens* seedlings at harvest 2 (after 20 weeks of waterlogging) in dolerite and mudstone soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

After 20 weeks of waterlogging, the effect of fertilisation on root:shoot ratio was non-significant (Table 3.2). The only significant variable was the effect of soil

type ($P = 0.0058$), with seedlings of both species, growing in dolerite having a higher root:shoot ratio than those in mudstone (Figure 3.14).

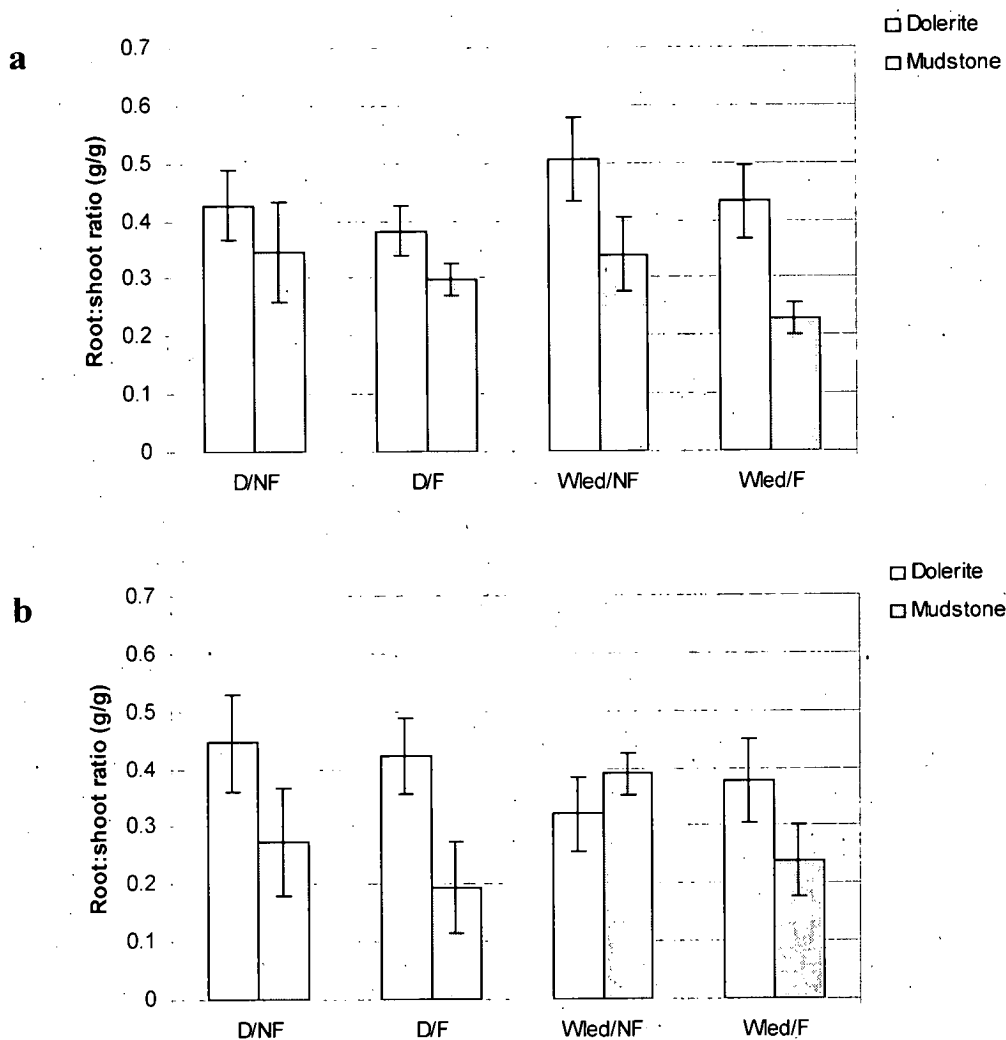


Figure 3.14 Mean root:shoot ratio of (a) *E. globulus* and (b) *E. nitens* seedlings at harvest 2 (after 20 weeks of waterlogging) in dolerite and mudstone soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

At the completion of 28 weeks of waterlogging (harvest 3), *E. nitens* had a significantly larger ($P = 0.0168$) biomass than *E. globulus* under all non-fertilised treatments (Figure 3.15). Fertilised plants generally had a higher total dry weight in both soil types, with no difference between the species. Waterlogged seedlings were significantly smaller ($P < 0.0001$) than drained seedlings (Figure 3.15).

There was also a significant ($P = 0.0007$) 3-way interaction between soil type,

fertilisation and waterlogging on total seedling dry weight (Table 3.3). This represents the difference in seedling dry weight between drained and waterlogged treatments in mudstone soil under fertilised conditions (Figure 3.15).

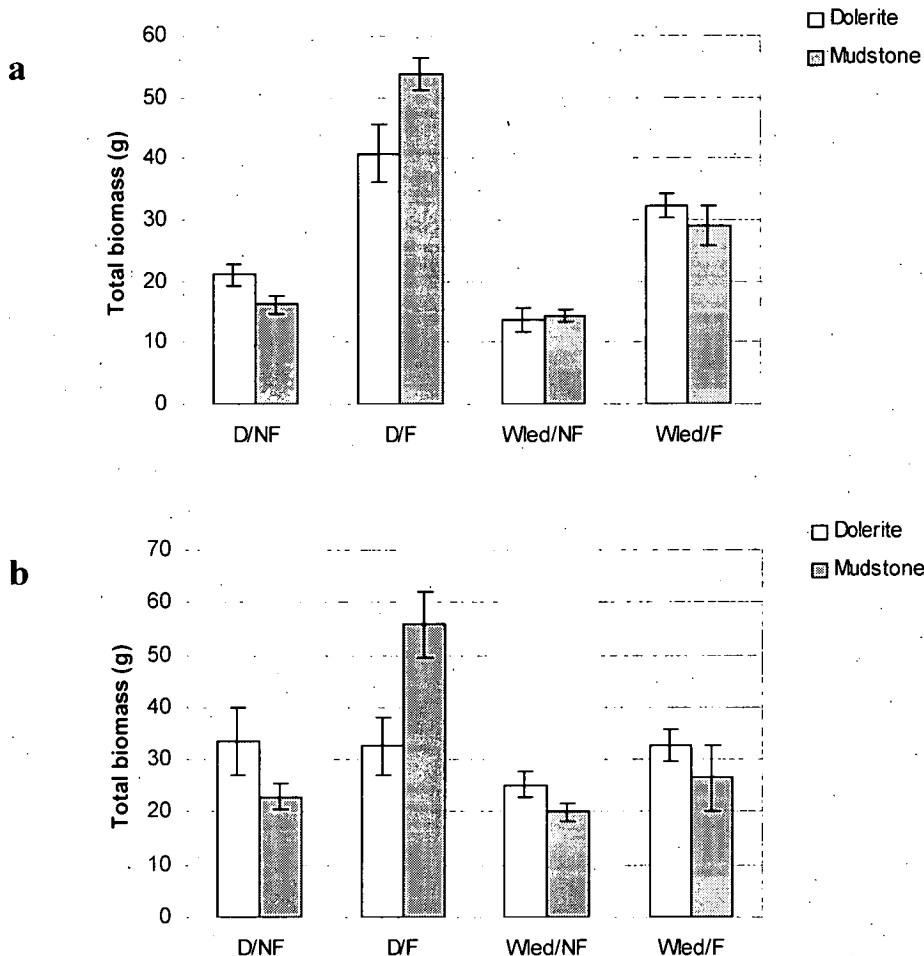


Figure 3.15 Total seedling dry weight (g) of *E. globulus* and *E. nitens* after 28 weeks of waterlogging at harvest 2 in dolerite (Dol) and mudstone (Mud) soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

The effects of fertilisation ($P = 0.0001$) and waterlogging ($P = 0.0001$) on stem diameter after 28 weeks (Figure 3.16) were the same as for 20 weeks of waterlogging (Figure 3.12). Similarly to total biomass results, there was a significant 3-way interaction between soil type, fertilisation and waterlogging ($P = 0.0098$) (Table 3.3).

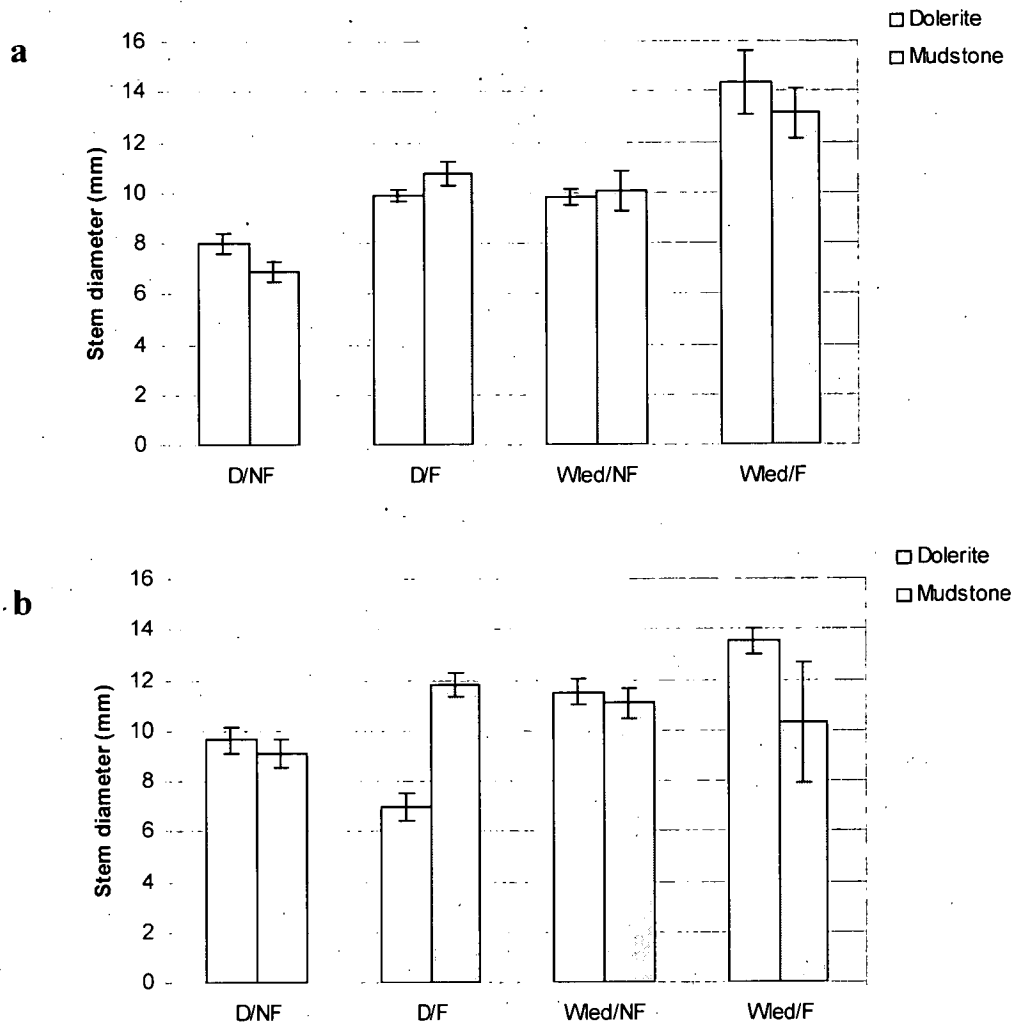


Figure 3.16 Mean stem diameter of (a) *E. globulus* and (b) *E. nitens* at harvest 3 (after 28 weeks of waterlogging) in dolerite and mudstone soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

After 28 weeks of waterlogging *E. globulus* had a significantly higher root:shoot ratio than *E. nitens* across all treatments ($P = 0.0014$, Figure 3.17). Waterlogged seedlings had significantly smaller ($P = 0.0391$) root:shoot ratios than drained seedlings, particularly in dolerite for *E. globulus* and mudstone for *E. nitens* seedlings. This effect was evident by the significant three-way interaction of species x soil type x waterlogging ($P = 0.0032$) (Table 3.3).

There was a significant increase in the root:shoot ratio of *E. globulus* seedlings between harvest 2 (Figure 3.14) and 3 (Figure 3.17).

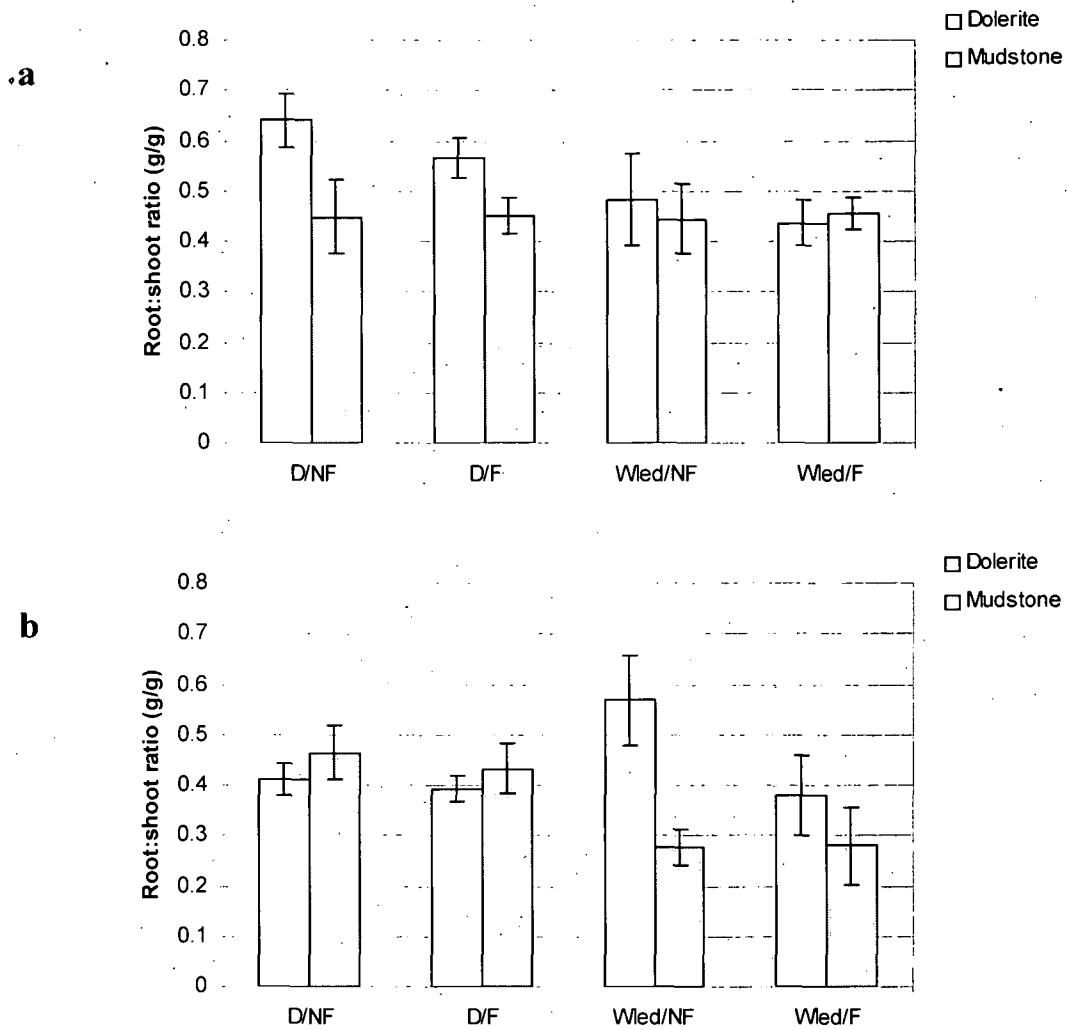


Figure 3.17 Mean root:shoot ratio of (a) *E. globulus* and (b) *E. nitens* seedlings at harvest 3 (after 28 weeks of waterlogging) in dolerite and mudstone soil types. Drained = D, waterlogged = Wled, fertilised = F and non-fertilised = NF. Bars represent standard error.

3.3.5 Leaf morphology

The degree of leaf loss in response to waterlogging was significantly different between species, indicated by a significant interaction between species and waterlogging ($P = 0.002$) on leaf number after 28 weeks of waterlogging (Table 3.3). *Eucalyptus nitens* had a significantly higher degree of observed leaf loss than *E. globulus* under waterlogged conditions.

3.3.6 Root morphology

There was no aerenchyma development observed prior to the onset of waterlogging. Twenty weeks of waterlogging had a significant effect on the development of aerenchymatous roots ($P = 0.005$) (Figure 3.18). *Eucalyptus globulus* produced only secondary aerenchymatous roots, in comparison, *E. nitens* displayed some capacity for the development of aerenchymatous root tips within the primary root system after prolonged exposure to waterlogging.

For *E. globulus* in dolerite soil, fertilised and waterlogged seedlings had double the dry weight of aerenchymatous roots than in other treatments. In mudstone, fertilised and waterlogged *E. nitens* had triple the aerenchyma of other treatments, but there was very high variability.

After 28 weeks of waterlogging, dolerite soil had significantly more aerenchymatous root development than mudstone ($P = 0.028$, Figure 3.19). There was no significant effect of species or fertilisation. During the 8 week period from harvest 2 to harvest 3, there was an increase in the percentage of aerenchymatous roots across most treatments. This was due to the death of primary roots and the growth of existing aerenchymatous roots. Fertilisation had the effect of shortening the length of the primary root system in both species across all treatments.

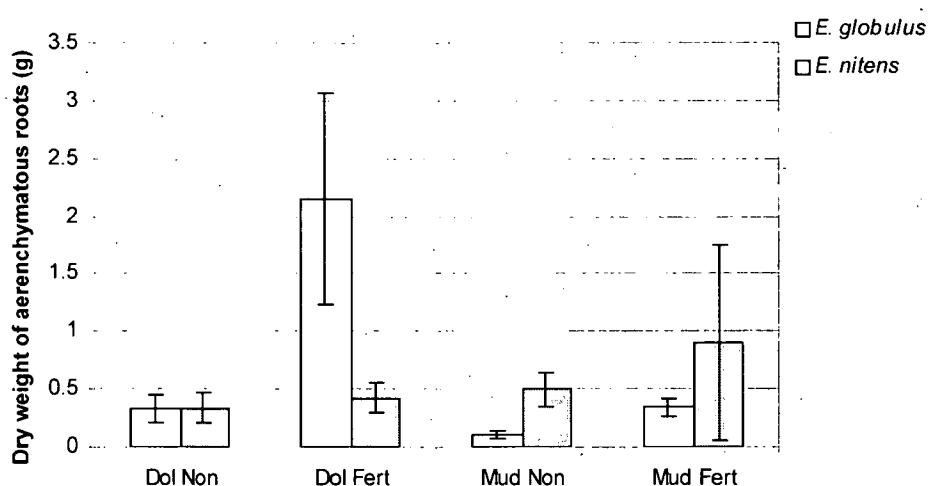


Figure 3.18 Dry weight percentage of aerenchymatous roots within the root system of waterlogged *E. globulus* and *E. nitens* seedlings at harvest 2 (after 20 weeks of waterlogging) in dolerite (Dol) and mudstone (Mud) soil. Non = non-fertilised and Fert = fertilised treatments. Bars represent standard error.

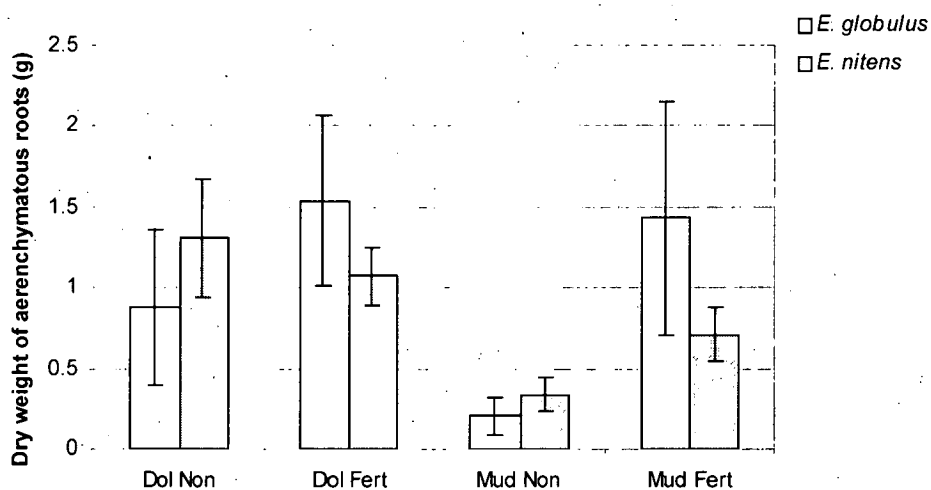


Figure 3.19 Dry weight percentage of aerenchymatous roots within the root system of waterlogged *E. globulus* and *E. nitens* seedlings at harvest 3 (after 28 weeks of waterlogging) in dolerite and mudstone soil. Non = non-fertilised and Fert = fertilised treatments. Bars represent standard error.

Table 3.1 Significance levels of species, soil type, fertilisation, waterlogging and the interactions of these on seedling height, biomass fresh (Fwgt) and dry (Dwgt) weights, leaf number and root:shoot ratio at harvest 1 (prior to waterlogging).

	Species	Soil	Fert	Water	Species x soil	Species x fert	Species x water	Soil x fert	Soil x water	Fert x water	Species x soil x water	Species x fert x water	Soil x fert x water	4-way
Initial height	0.0001	0.038	0.007							0.0136			0.01	
Final height	0.049	0.0002	0.0001					0.002			0.0386			
Stem diameter	0.0001	0.0001	0.0001					0.047						
Leaf Fwgt	0.006	0.007	0.0001					0.0019						
Leaf Dwgt	0.0003	0.0178	0.0001					0.0011						
Leaf number	<0.0001		<0.0001											
Root Dwgt	0.005	0.0001	0.018											
Total Dwgt	<0.0001	<0.0001	<0.0001					<0.0001						
Root:shoot		0.0035	0.0001	0.0069				0.0027						

Table 3.2 Significance levels (*P*) of species, soil type, fertilisation, waterlogging and the interactions of all these on the height, biomass fresh (Fwgt) and dry weights (Dwgt), stele diameter, percentage stem cortex, leaf number, percentage biomass of aerenchymatous roots and root to shoot ratio at Harvest 2 (20 weeks waterlogging).

	Species	Soil	Fert	Water	Species x soil	Species x fert	Species x water	Soil x fert	Soil x water	Fert x water	Species x soil x water	Species x fert x water	Soil x fert x water	4-way
Initial height	0.0001													
Final height		0.018	<0.0001	0.002				0.0004	0.019			0.006		
Stem diameter			0.0001	0.0001		0.002								
Leaf Fwgt	0.00001	0.003	<0.0001			<0.0001				0.041				
Leaf Dwgt	0.0002	0.002	0.0001			0.0001		0.019		0.017				
Stele diameter	0.001		0.0001			0.018			0.029				0.0001	
% cortex	0.002			0.0001										
Leaf number	<0.0001	0.016	<0.0001			0.041								
Root Dwgt		0.016	<0.0001	0.0007		0.0011	0.017		0.008	0.008			0.0016	0.0032
% Aer				0.005	0.01									
Aer Dwgt				0.005	0.017					0.039				
Total Dwgt			<0.0001			<0.0001		0.0002					0.028	0.024
Root:shoot		0.006												

Table 3.3 Significance levels (*P*) of species, soil type, fertilisation, waterlogging and the interactions of all these on the height, biomass fresh (Fwgt) and dry weights (Dwgt), stele diameter, percentage stem cortex, leaf number, percentage biomass of aerenchymatous roots and root to shoot ratio at Harvest 3 (28 weeks waterlogging).

	Species	Soil	Fert	Water	Species x soil	Species x Fert	Species x water	Soil x fert	Soil x water	Fert x water	Species x soil x water	Species x fert x water	Soil x fert x water	4-way
Initial height	0.0001				0.031			0.047						
Final height		0.0015	<0.0001	<0.0001			0.048	0.046	0.0002	0.028			0.041	
Stem diameter			0.0001	0.0001		0.006							0.0098	
Stem Fwgt	0.0103		0.0001	0.0002		0.018		0.002	0.0008	0.013			0.0001	
Stem Dwgt	0.0034		0.0001	0.0008		0.041		0.004	0.0022	0.006			0.0001	
Leaf Fwgt		0.0485	0.0001	0.0001		0.018								
Leaf Dwgt		0.0195	0.0001	0.0001										
Stele diam	0.001		<0.0001			0.0003							0.0105	
% cortex				0.0001										
Leaf number		0.0096	<0.0001	<0.0001			0.0024							
Root Dwgt			<0.0001	<0.0001		0.0009		0.009			0.008		0.028	
% Aer		0.0287		0.0001					0.041					
Aer Dwgt				0.00001										
Total dwgt	0.0168		<0.0001	<0.0001		<0.0001		0.0067					0.0007	
Root:shoot	0.0014	0.0272		0.0391							0.0032			

3.3.6 Physiology

Prior to the imposition of waterlogging (April 2003, Figure 3.20) fertiliser application increased the photosynthetic rate of both *E. nitens* and *E. globulus* ($P = 0.0012$ and 0.00085) and the stomatal conductance of *E. globulus* ($P = 0.033$). There was no effect of fertilisation on predawn leaf water potential.

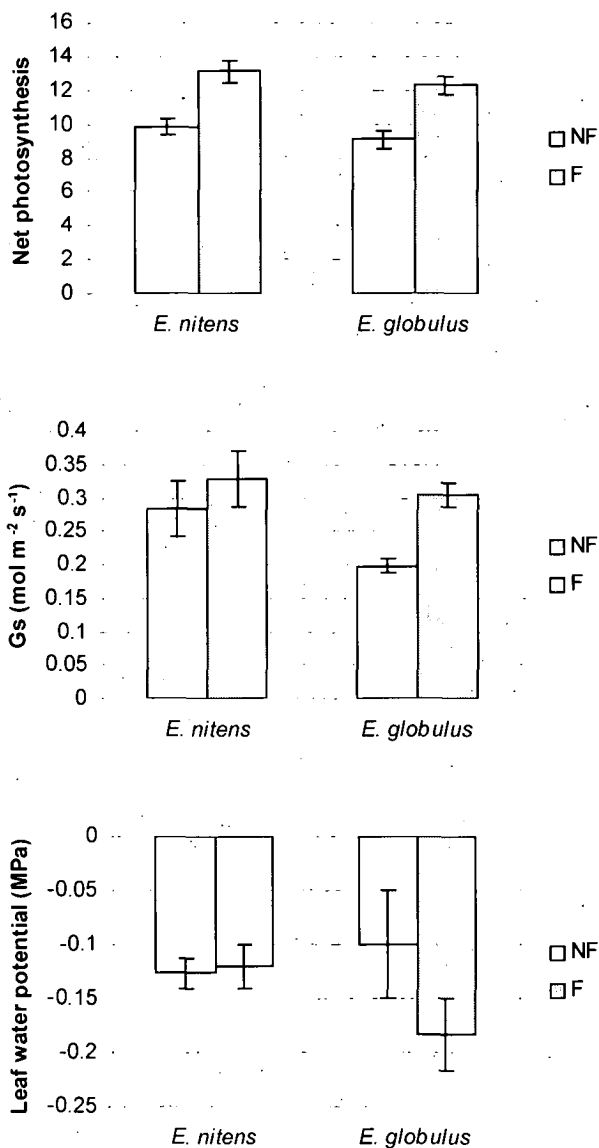


Figure 3.20 Net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) and predawn leaf water potential (MPa) of Non-fertilised (NF) and Fertilised (F) *Eucalyptus nitens* and *E. globulus* (right) prior to waterlogging (April 2003). Bars represent standard error.

Significantly reduced photosynthetic rates and stomatal conductance in response to waterlogged conditions first became evident after three weeks (May, see Table 3.4) in *E. globulus*, and were maintained throughout the remainder of the 28 weeks of waterlogging (Figure 3.21). The F_v/F_m ratio of waterlogged *E. globulus* seedlings decreased to < 0.73 after 17 weeks of waterlogging. Although this was a significant reduction in photosynthetic efficiency, it was not indicative of photoinhibition (see Appendix 4).

At 28 weeks waterlogging (November), there was significantly reduced net photosynthesis ($P < 0.0001$), stomatal conductance ($P < 0.0001$) and photosynthetic efficiency ($P < 0.0001$) of *E. globulus* seedlings (Table 3.5, Figure 3.21). There was no effect on leaf water potential. These trends were less obvious in *E. nitens*.

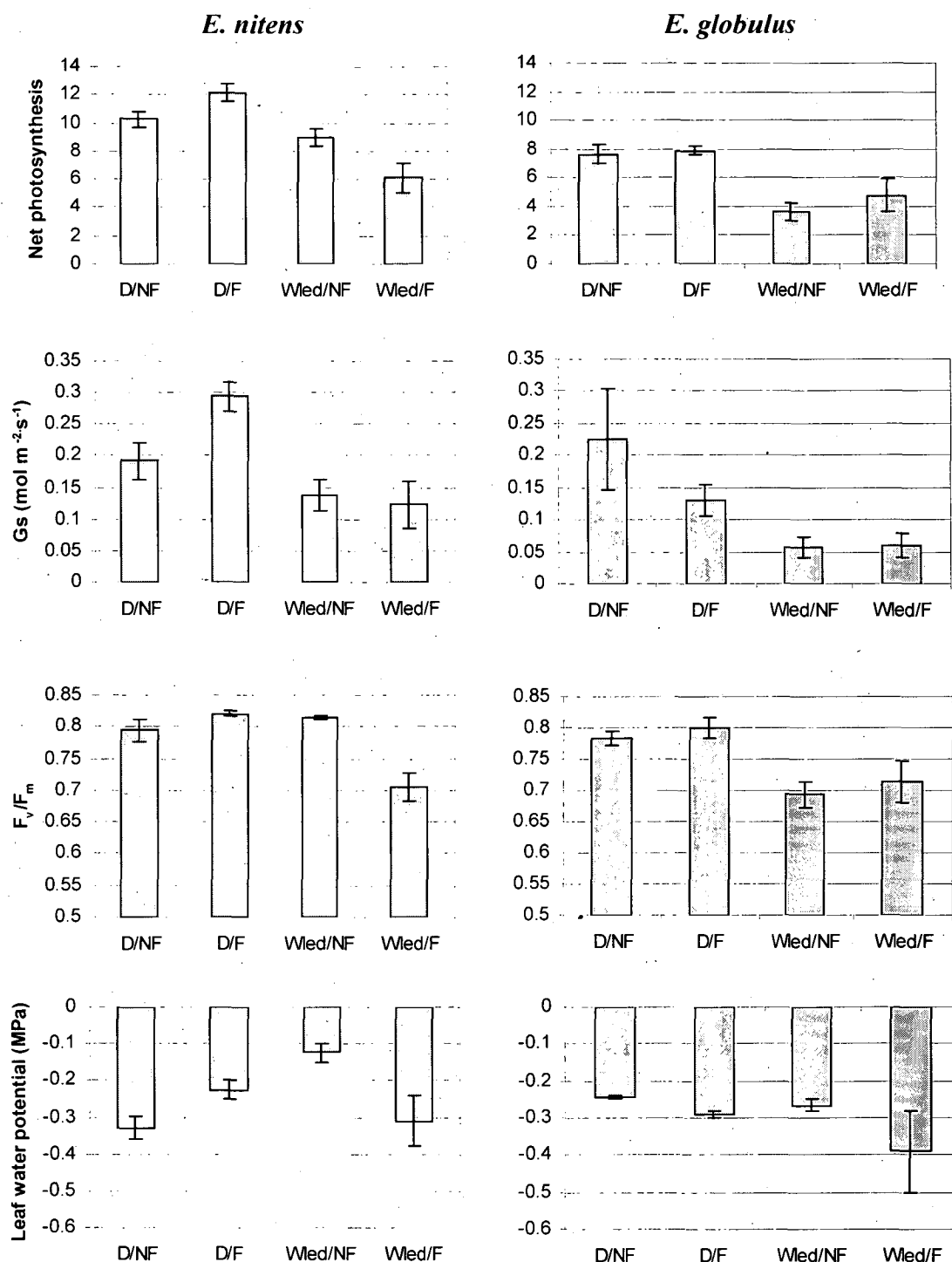


Figure 3.21 Net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$), predawn F_v/F_m and predawn leaf water potential (MPa) of drained (D), waterlogged (Wled), non-fertilised (NF) and fertilised (F) *Eucalyptus nitens* (left) and *E. globulus* (right) seedlings at harvest 3 after 28 weeks of waterlogging. Bars represent standard error.

Table 3.4 Significance levels (*P*) of species, soil type, fertilisation, waterlogging and the interactions of all these on the photosynthetic rate (photo rate), stomatal conductance (Gs), predawn leaf water potential and photoefficiency (Fv/Fm) of seedlings in May, June and July

	Species	Soil	Fert	Water	Species x soil	Species x Fert	Species x water	Soil x fert	Soil x water	Fert x water	Species x soil x water	Species x fert x water	Soil x fert x water	4-way
MAY														
Photo Rate	0.0001		0.0001	0.0007			0.0120							
Gs	0.0001		0.0033	0.0001		0.0303	0.0004					0.0213		
Water potential	0.0055													
Fv/Fm	0.0161			0.0401										
JUNE														
Photo Rate														
Gs														
Water potential						0.0002								
Fv/Fm	0.0316					0.0159	0.0001			0.00001				
JULY														
Photo Rate	0.0001		0.0488	0.0001			0.0163							
Gs	0.0001		0.0072	0.0001								0.0213		
Water potential	0.0055													
Fv/Fm	0.0001									0.0025				

Table 3.5 Significance levels (*P*) of species, soil type, fertilisation, waterlogging and the interactions of all these on the photosynthetic rate (photo rate), stomatal conductance (Gs), predawn leaf water potential and photoefficiency (Fv/Fm) of seedlings in September and November.

	Species	Soil	Fert	Water	Species x soil	Species x Fert	Species x water	Soil x fert	Soil x water	Fert x water	Species x soil x water	Species x fert x water	Soil x fert x water	4-way
SEPT														
Photo.Rate	0.0004			0.0001			0.0034							
Gs	0.0001			0.0001			0.0018			0.0045				
Water potential				0.0235										
Fv/Fm	0.0001			0.0001			0.003							
NOV														
Photo Rate	0.0001			0.0001								0.0106		
Gs	0.0108			0.0001								0.0429		
Water potential									0.0293					
Fv/Fm	0.0067			0.0001		0.0263			0.0135			0.0105		

3.3.7 Pigment analysis

Prior to the onset of waterlogged conditions, *E. globulus* had significantly higher concentrations of neoxanthin, lutein, β -carotene, violoxanthin, zeaxanthin, total xanthophylls and chlorophyll than *E. nitens* (Table 3.6). There was a significant positive effect of fertilisation on the concentration of chlorophyll ($P = 0.05$) and a negative effect on the xanthophyll conversion ratio (az/vaz) ($P = 0.0210$) (Figure 3.22). There was no interaction between species and fertilisation prior to the onset of waterlogged conditions, indicating that the two species were affected in the same way (Table 3.6).

Table 3.6 Significance levels (P values < 0.05) of the variables species, fertilisation and their interaction on the concentration of foliar pigments in April 2003 prior to the onset of waterlogging.

Pigment	Species	Fert	Spp x Fert
Neoxanthin	0.0059		
Lutein	0.0026		
B-carotene			
Violoxanthin	0.0232		
Antheroxanthin			
Total xanthophylls	0.0043		
Xanthophyll conversion ratio (az/vaz)	0.0300	0.05	
Chlorophyll	0.0022	0.0210	
Zeaxanthin			

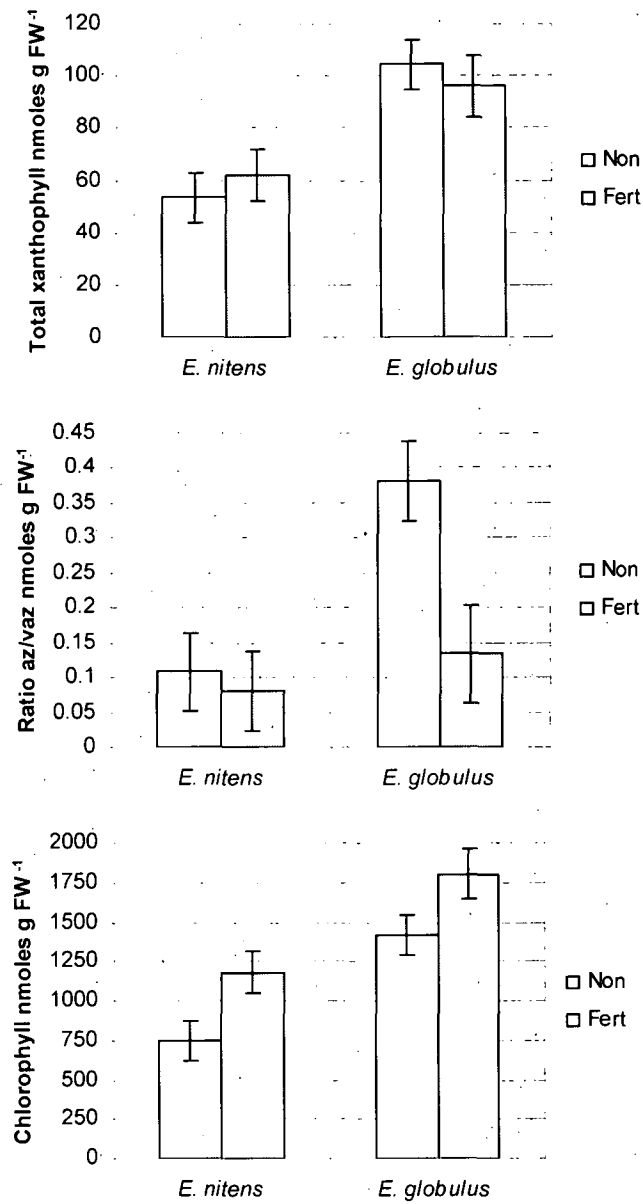


Figure 3.22 Foliar concentrations (nmoles/g fresh weight) of total xanthophyll pool, xanthophyll conversion ratio and chlorophyll in *E. nitens* and *E. globulus* in April 2003 prior to the onset of waterlogging. Bars represent standard error.

After 28 weeks of waterlogging, *E. globulus* had significantly higher concentrations of antheroxanthin, zeaxanthin and xanthophyll conversion ratio than *E. nitens* (Table 3.7). Fertiliser had a significant effect on total xanthophyll pool size ($P < 0.005$, Table 3.7). There were significant 3-way interactions on neoxanthin, lutein, β -carotene, violoxanthin and chlorophyll (Table 3.7).

Eucalyptus nitens was more sensitive to waterlogging stress than *E. globulus*, with increased xanthophyll pigment concentration. *Eucalyptus globulus* was more sensitive to fertilisation (Figure 3.23).

Table 3.7 Significance levels (P values < 0.05) of the variables species, fertilisation and their interaction on the concentration of foliar pigments after 28 weeks of waterlogging in November 2003.

Pigment	Species	Fert	Water	Spp x Fert	Spp x Water	Fert x Water	3-way
Neoxanthin			0.0405				0.0306
Lutein							0.0315
B-carotene							0.0324
Violoxanthin	0.0449						0.0195
Antheroxanthin	0.0079	0.0066	0.0213	0.0190			
Total xanthophylls		0.0049		0.00495			
Xanthophyll conversion ratio (az/vaz)	<0.0001			0.0188			
Chlorophyll							0.0301
Zeaxanthin	0.0271						
Xanthophyll / Chlorophyll	<0.0001	0.0219	0.044	0.0127			

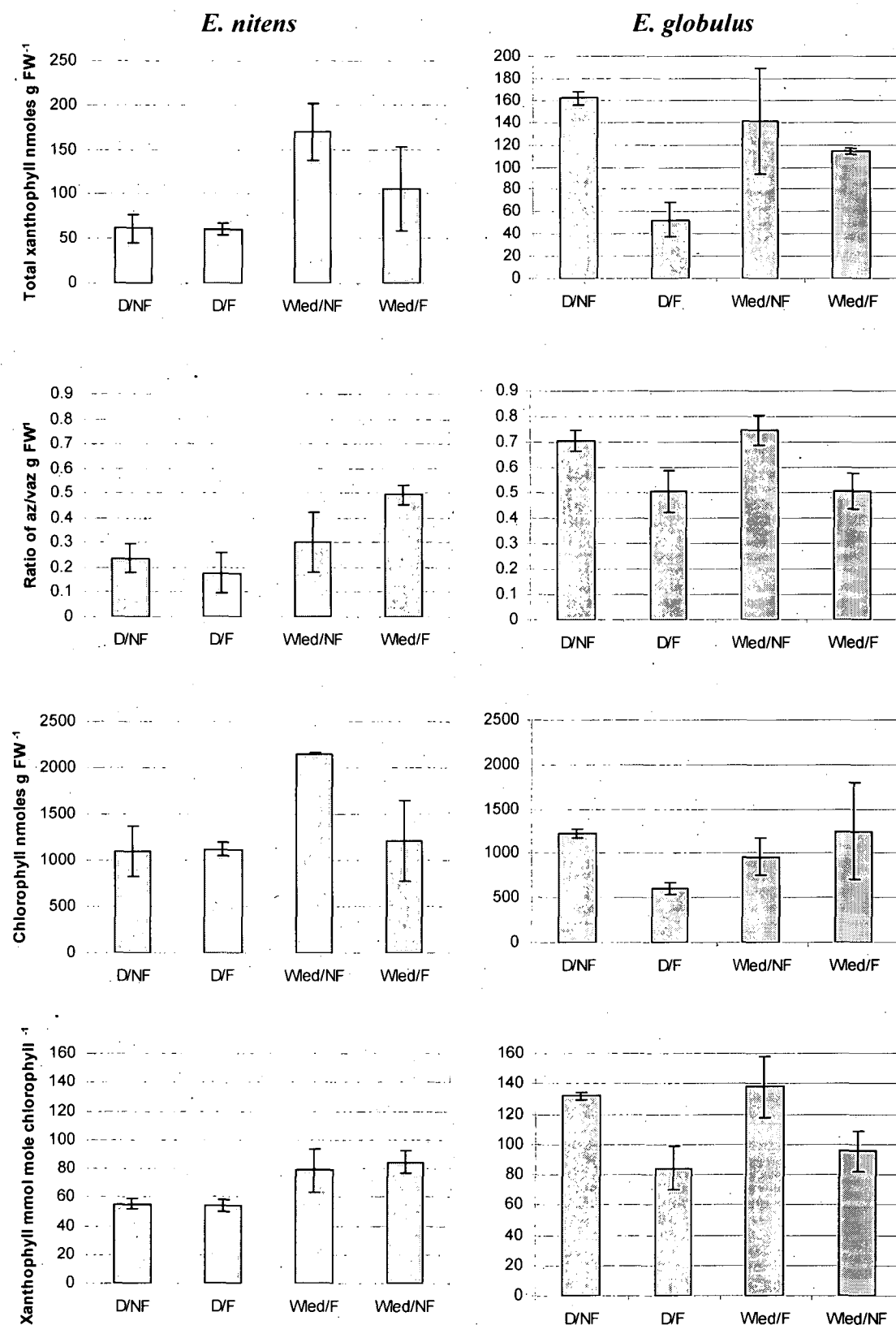


Figure 3.23 Foliar concentrations (nmol/g fresh weight) of total xanthophyll pool, xanthophyll conversion ratio, chlorophyll and mmol of total xanthophyll per mole chlorophyll in *E. nitens* (left) and *E. globulus* (right) after 28 weeks of waterlogging. Bars represent standard error.

3.3.8 Nutrition analysis

Eucalyptus globulus had significantly higher concentrations of potassium, magnesium and calcium than *E. nitens* after 28 weeks of waterlogging (Table 3.8, Figure 3.24). Waterlogged plants had significantly higher foliar concentration of nitrogen than drained plants ($P < 0.0001$). Waterlogging resulted in a significant decrease in the foliar concentrations of phosphorus ($P = 0.0063$), potassium ($P = 0.0415$) and calcium ($P < 0.0001$, Table 3.8) in both species.

Fertiliser significantly increased ($P = 0.0001$) foliar phosphorus concentration in waterlogged and drained plants (Figure 3.24).

There was a significant 3-way interaction between species, fertilisation and waterlogging on the concentration of nitrogen ($P = 0.0329$) and magnesium ($P = 0.0148$). *Eucalyptus nitens* had reduced foliar concentrations under fertilised and waterlogged treatments, whereas, *E. globulus* had an increase in the foliar concentration of these two elements in response to fertilisation under waterlogged conditions (Figure 3.24).

Table 3.8 Significance levels (P values < 0.05) of species, fertilisation and waterlogging on the concentration of foliar nitrogen, phosphorus, potassium, magnesium and calcium after 28 weeks of waterlogging.

Element	Spp	Water	Spp x Water	Fert	Spp x Fert	Water x Fert	3-way
Nitrogen		<0.0001					0.0329
Phosphorus		0.0063		0.0001			
Potassium	0.0006	0.0415					
Magnesium	<0.0001		0.0004				0.0148
Calcium	<0.0001	<0.0001					

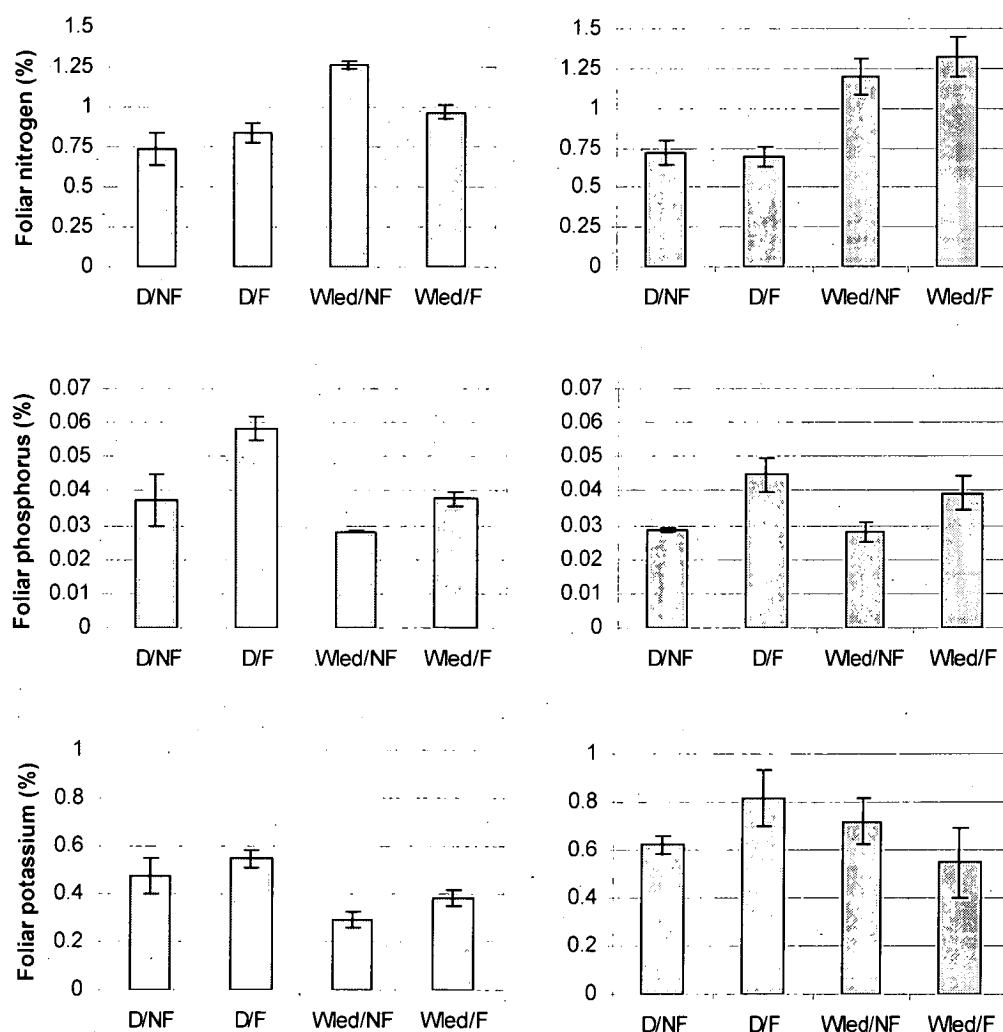


Figure 3.24 Foliar concentrations (%) of nitrogen, phosphorus and potassium in fertilised (F) and non-fertilised (NF) *E. nitens* and *E. globulus* (right) after 28 weeks of waterlogging (November 2003). Bars represent standard error.

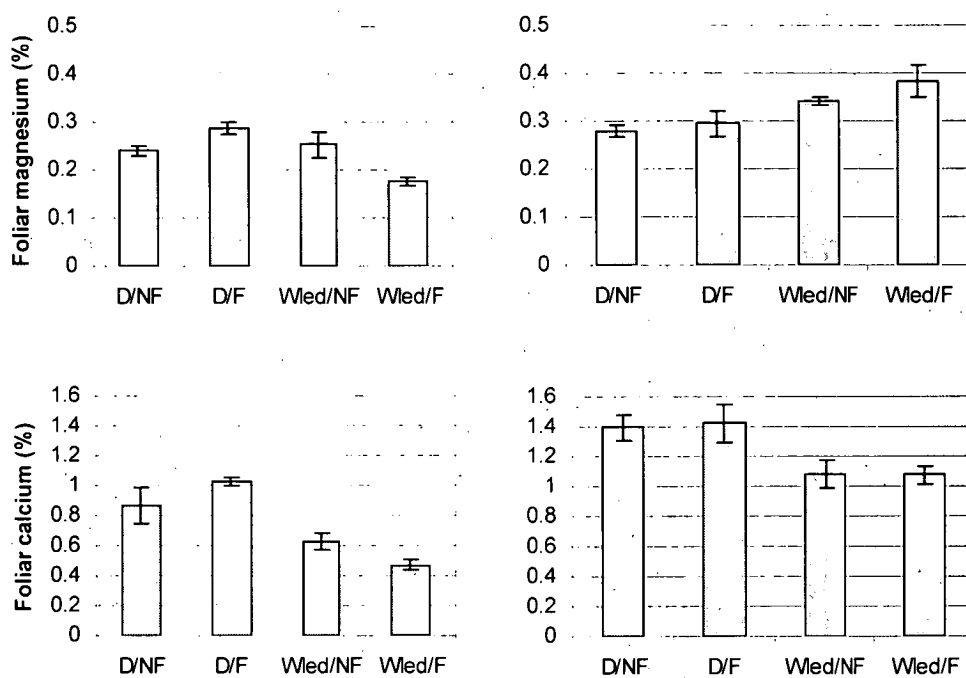


Figure 3.24 (continued) Foliar concentrations (%) of magnesium and calcium in fertilised (F) and non-fertilised (NF) *E. nitens* and *E. globulus* (right) after 28 weeks of waterlogging (November 2003). Bars represent standard error.

3.4 Discussion

The use of dolerite and mudstone soils as substrates in this experiment allowed examination of the effect of soil type on the development of waterlogging stress and the capacity for morphological adaptation to waterlogging. Soil organic matter content, cation exchange capacity, buffering capacity, texture and microbial biomass components are important factors which affect the depletion of soil DO and changes in pH and Eh under waterlogged conditions (Phillips and Greenway 1999). The rapid decline in soil pH after the imposition of waterlogged conditions was observed in both soil types. This early decline in pH, and then slow increase to near neutral values has been reported by Janiesch (1991) and Ponnampetuma (1972). The decrease in pH from 7 to 5 can result in reductions in the relative availabilities of nitrogen, sulphur, calcium, magnesium, molybdenum and phosphorus (McLaren and Cameron 1996). This lowering of the soil pH may be caused by leaching of basic cations (e.g. calcium, magnesium and potassium) (McLaren and Cameron 1996).

The oxygen concentration required to maintain cellular function and root growth is dependent on species. In wheat, the critical oxygen concentration for respiration in the expanding root tip averaged < 1 ppm, which was significantly higher than that recorded for rice (Thomson et al. 1990). After 49 days of waterlogging, the dissolved oxygen (DO) of waterlogged dolerite soil was 2.5 ppm but < 1 ppm in mudstone soil, which is nearing critical minimum oxygen concentrations for root respiration. The difference between the two soil types in the final DO concentration may be attributable to the textural properties of the two soils. Highly compacted waterlogged soils do not contain as many oxic sites as loosely packed waterlogged soils (Engelaar et al. 2000).

As the concentration of oxygen decreases, due to slow rates of diffusion and high rates of oxygen consumption (by microbial and root respiration), the redox potential (Eh) of the soil solution declines. This reduction in soil Eh can occur rapidly, and has been reported to develop within 24 hours of waterlogging (Pezeshki and Chambers 1984). The reduction in soil redox potentials to below

100 mV is indicative of reducing conditions (Patrick and Mahapatra 1968). In this experiment, the reduction of soil redox potentials to less than 90 mV in dolerite and less than 30 mV in mudstone soil after 49 days of waterlogging will result in a reduction in the availability of nitrate, iron and sulphate (Bohn et al. 1979).

Under glasshouse conditions, there was 100 % survival of *E. globulus* and *E. nitens* seedlings to 28 weeks of waterlogging. Despite this high level of survival, waterlogging significantly reduced seedling growth in height and biomass. Fertiliser was effective in increasing the height growth of waterlogged *E. globulus* and *E. nitens* seedlings over the initial 100 days of waterlogging, which is evidence of the short term effect of pre-waterlogging fertilisation, and potential requirement for a secondary or long term application method.

Control of growth rate is an adaptation to and indicator of tolerance of seedlings exposed to environmental stress (Grove et al. 1996). Differences in growth pattern under waterlogged conditions were demonstrated by the two species. *Eucalyptus globulus* maintained a relatively high rate of growth throughout waterlogging. This is consistent with the results of Connell (1998), with *E. nitens* exhibiting a greater reduction in growth rate under waterlogged conditions. *Eucalyptus nitens* had an immediate growth response to the application of waterlogged conditions with a decrease in growth rate, which was maintained throughout the 28 week waterlogging period.

The degree and extent of aerenchyma development is indicative of the level of tolerance of a species to waterlogging, though root aeration traits should not be used solely as the means of tolerance assessment (Garthwaite et al. 2003). Waterlogging induced the formation of aerenchyma in both *E. nitens* and *E. globulus*, with the development of aerenchymatous roots and stem hypertrophy in both species. *Eucalyptus globulus* had significantly higher percentage of stem cortex than *E. nitens*, indicative of a greater capacity for stem hypertrophy in response to waterlogging. This was also evidenced by the greater stem diameter

in *E. globulus* under waterlogged and fertilised conditions. *Eucalyptus globulus* had significantly fewer aerenchymatous roots in the mudstone soil, compared to *E. nitens*. This may be due to textural constraints of the soil as previously described and/or root size. At the conclusion of the waterlogging treatment, seedlings of both species exhibited extensive primary root death, with an increase in the root:shoot ratio and in the relative percentage of aerenchymatous roots. The development of aerenchyma within the primary root system, characterised by the appearance of swollen root tips, was only observed in waterlogged *E. nitens* seedlings. This indicates that *E. nitens* has an inherently greater capacity for the development of root aerenchyma under waterlogged conditions than *E. globulus*, which is contrary to the conclusions of Connell (1998).

Rapid stomatal closure and reduction in net photosynthesis are common responses to waterlogging in all plant species, regardless of tolerance (Kozłowski 1997). This response was observed in waterlogged *E. globulus* seedlings after three weeks of waterlogging, with a significant depression in net photosynthesis and stomatal conductance. As the species maintains a relatively high rate of growth during early exposure to waterlogging, this indicates that the species allocates all of its resources to the maintenance of growth. In comparison, *E. nitens* did not exhibit reduced physiological performance until after 28 weeks of waterlogging. As the species demonstrates an early reduction in growth rate under waterlogged conditions, whilst maintaining a high rate of photosynthesis, it may be indicative of the allocation of resources into the development of protective and adaptive features.

The reduction in photosynthesis after prolonged exposure to waterlogging is often attributable to inhibitory effects on the photosynthetic process (Kozłowski 1997). Photoinhibition of waterlogged *E. nitens* seedlings was reported by Close and Davidson (2003) after eight months of waterlogging. However, there was no depression in F_v/F_m ratio of waterlogged seedlings in this experiment, with all seedlings maintaining high photosynthetic efficiency (F_v/F_m of > 0.7) (Demmig and Bjorkman 1987).

The total xanthophyll composition was significantly higher under waterlogged conditions in *E. nitens* and under non-fertilised conditions in *E. globulus*. This is consistent with the results of Close and Davidson (2003) with an increase in the xanthophyll pigment content and xanthophyll-cycle conversion ratio of waterlogged *E. nitens*. This is indicative of sustained xanthophyll engagement and represents a photoprotective mechanism in *E. nitens*. In *E. globulus* the development of epicuticular wax on new growth may provide a similar protection.

Neither of the species exhibited reduced predawn water potentials at any time during the waterlogging period. This is consistent with reports that many eucalypt species, including *E. camaldulensis*, *E. globulus* and *E. nitens*, do not develop water stress in response to waterlogged conditions (Pereira and Kozlowski 1977, Close and Davidson 2003). This result supports the hypothesis that the physiological and growth response of waterlogged eucalypt species is induced by mechanisms other than the development of plant water stress (Pereira and Kozlowski 1977, Sena Gomes and Kozlowski 1980).

The development of nutrient deficiency under waterlogged conditions is one potential mechanism responsible for the development of waterlogging stress (Kozlowski 1997). The reduced uptake of nitrogen, phosphorus, potassium and calcium can occur rapidly in response to the development of waterlogged conditions (Kozlowski 1997, Van der Moezel et al. 1988, Drew and Sisworo 1979) and may be the primary cause of waterlogging injury (Topa and Cheeseman 1992). The severity of waterlogging damage has been reported to depend on the season and rate of plant growth, indicating that waterlogging induces source deficiencies within the plant (Hook et al. 1983, Angelov et al. 1996). Deficiency in essential plant nutrients and changes in the ratio of available nutrients results in changes to plant biomass allocation patterns, pigment composition and the development of photoinhibition which may subsequently affect the growth and productivity of waterlogged plants.

All treatments exhibited foliar nutrient concentrations within the range of other published results. These are; nitrogen (1-2%), phosphorus (0.07-0.13%), calcium (0.75-2%), magnesium (0.18-0.23%) and potassium (0.55-0.7), with the two species displaying very similar concentrations (Hawkins and Polgase 2000, Saur et al 2000). Waterlogging resulted in a significant decrease in the foliar concentrations of phosphorus, potassium and calcium in both eucalypt species grown in dolerite soil. This is consistent with the results of Close and Davidson (2003). Reduced foliar nutrient status is not necessarily indicative of a functional deficiency, but reduced nutrient levels in waterlogged seedlings relative to drained controls does indicate reduced nutrient uptake under waterlogged conditions, and the potential for the development of foliar nutrient deficiencies.

Foliar phosphorus deficiency can lead to decreased electron flow and associated inhibition of photosynthesis (Plesnicar et al. 1994), and the lowered levels in plants in this experiment may explain the reduction in net photosynthesis observed in waterlogged seedlings. Fertilisation had a significant positive effect on the growth of both species under waterlogged conditions, and increased the physiological performance under waterlogged conditions over the initial few weeks of waterlogging.

There was a significant increase in foliar nitrogen concentration of waterlogged seedlings. This result may be due to the re-translocation of nitrogen from old leaves and the accumulation of the element in the newly developing foliage (Dell et al. 2001) which was harvested for analysis. Therefore, the nutrient analysis conducted during this experiment may underestimate the degree of whole plant deficiency, especially for phloem-mobile elements including nitrogen, phosphorus, potassium and magnesium (Dell et al. 2001). The foliar concentration of elements needs to be considered in relation to the total leaf area of waterlogged seedlings.

In response to nutrient deficiency, there are a number of physiological adaptations that plants may display. These include the regulation of growth rate, increased

efficiency of nutrient utilisation through biochemical cycling, increased nutrient retention (Grove et al. 1996) and the increased allocation of resources to root growth (Grove et al. 1996, Marschner et al. 1996). As discussed, *E. nitens* demonstrates regulation of growth during the early stages of waterlogging and relatively high rates of leaf loss, which may be adaptive responses to the development of nutrient deficiency.

The re-translocation of foliar nutrients in eucalypt species is an important process, with a high proportion of nitrogen and phosphorus remobilised during senescence (Grove et al. 1996). The re-distribution of nutrients from old senescing leaves to newly expanding leaves may provide a mechanism for the allocation of deficient elements into actively growing zones to increase the survival of waterlogged seedlings. *Eucalyptus nitens* was observed to have a higher rate of mature leaf necrosis, senescence and abscission than *E. globulus*, which is demonstrated by the significant interactive effect of species and waterlogging on the mean leaf number of seedlings. The inherently higher nutrient status of *E. globulus* results in a greater pool of nutrients available for re-translocation, which may be responsible for the lower degree of leaf abscission than *E. nitens* (Close and Beadle 2004). This substantial leaf loss in *E. nitens* may explain the high rates of photosynthesis and high foliar chlorophyll concentrations maintained during the waterlogging treatment.

Eucalyptus globulus was more responsive to fertiliser addition than *E. nitens*. In contrast, *E. nitens* displayed a greater susceptibility to waterlogging in terms of growth and physiology. This seemingly greater sensitivity to waterlogging in terms of growth and development of photoprotective pigments indicates that *E. nitens* has an inherently greater capacity for adaptation to waterlogging than *E. globulus*.

The amelioration of foliar nutrient deficiency and growth reduction in waterlogged *E. globulus* and *E. nitens* through the application of fertiliser prior to waterlogging was partly successful. These results indicate that the amount, timing

and mode of fertiliser application are factors which should be investigated in relation to minimising the loss in productivity of waterlogged *E. globulus* and *E. nitens*.

~ CHAPTER 4 ~

The effect of fertiliser mode and timing of application in alleviating waterlogging damage

4.1 Introduction

The oxygen within the soil solution of waterlogged soil is rapidly consumed through respiration by plant roots, bacteria and other microbes. The low rate of gaseous diffusion in water prevents replenishment of soil oxygen. Soil redox potential decreases as oxygen concentrations decrease, and other elements than oxygen are utilised as electron acceptors and become reduced (Phillips and Greenway 1998). The rate and magnitude of changes in dissolved oxygen concentration, redox potential and pH is dependent on the rates of aerobic respiration and by soil type and climate, particularly; (i) soil organic matter content and (ii) concentration of NO_3^- (Phillips 1999). Soil redox values below +330 mV can be indicative of developing hypoxic conditions (reduction in oxygen) (Engelaar et al. 2000). A substantial drop in redox potential will result in changes to the availability of essential plant nutrients. The sequential reduction of oxygen; $\text{O}_2 + 4\text{H}^+ + 4e^- \rightarrow 2\text{H}_2\text{O}$

nitrate; $\text{NO}_3^- + 2\text{H}^+ + 2e^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$

manganese; $\text{Mn}^{3+} + e^- \rightarrow \text{Mn}^{2+}$

iron; $\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}$, and

sulphate; $\text{SO}_4 + 10\text{H}^+ + 8e^- \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O}$ occurs as the redox potential drops, with each step buffered until exhaustion of the preceding element. Therefore, changes in the nutrient status of a soil are strongly dependent on the concentration of these elements.

Mobile mineral elements move through the soil by both convective (mass flow) and diffusive processes (Clarkson and Hanson 1980). The uptake of available nutrients by plant roots is dependent on the absorption capacity of the root, root surface area and by the mobility of ions within the soil matrix (Escamarrilla and

Comerford 1998). Nitrate, sulphate, calcium and magnesium are highly mobile compounds, whilst phosphorus, zinc and copper are immobile, requiring active soil exploration for sufficient uptake (Grove et al. 1996).

In conjunction with the loss in available nutrients under waterlogged conditions, the rate of nutrient uptake from the soil by plant roots can also be affected. The timing and direction of root growth and root longevity, and therefore the volume of soil explored by roots, is controlled by environmental factors including soil temperature, moisture and availability of nutrients (Gill and Jackson 2000). Root tip death under anoxic conditions may impair the uptake and transport of nutrients into and within the root tissue. Waterlogging has been reported to reduce the effective absorption area of plant roots (Yang et al. 2004), which is a major factor affecting nutrient acquisition by the plant. The physical segregation of absorption and translocation of specific elements along the root length (Clarkson and Hanson 1980) will be affected by root death. Therefore, the typical morphological changes to the root system of waterlogged plants may reduce the capacity of the roots to explore for, uptake and transport nutrients.

There are potentially two energy-dependent steps in the transport of solutes within the root (Gibbs et al. 1998). The first is the transport across the epidermal layer, equivalent to uptake from the soil, and the second is transport of solutes into the pericycle region of the stele for translocation in the xylem. As the energy yield from anaerobic respiration is only 2 mole ATP per mole hexose, compared to 36 mole ATP per mole hexose by aerobic respiration, there is a reduction in the energy status of root tissue. Therefore the effectiveness of the two energy-dependent processes of nutrient uptake and transport within the root may be reduced. Ineffective uptake may lead to nutrient deficiency within the plant despite nutrients being readily available within the soil solution.

Nutrient deficiency affects biomass partitioning, photosynthetic capacity and the export of photosynthates, which in turn affects growth. The development of waterlogging-induced nutrient deficiency has been reported in a number of species; nitrogen in soybean (Bacanamwo and Purcell 1999) and wheat (Malik et

al. 2001), phosphorus, potassium, calcium and magnesium in loblolly pine (Hook et al. 1983). A significant reduction in potassium and calcium concentrations was reported in six eucalypt species by Van der Moezel et al. (1988). More recently, a study by Close and Davidson (2003) reported significant reductions in foliar calcium, potassium, phosphorus and in stem calcium concentrations in waterlogged *E. nitens* saplings.

The reduction in foliar nutrient status has been reported to develop very quickly in waterlogged plants (within 2 days of the onset of waterlogged conditions) (Drew and Sisworo 1979). The relative role of reduced soil availability and the reduced capacity for root uptake of nutrients are the two mechanisms involved in the development of plant nutrient deficiency under waterlogged conditions. The timing of the initiation of physiological damage within the plant has been used to determine the relative role of these two factors in the development of nutrient deficiency in waterlogged plants. The uptake of ions by excised roots and whole plants in solution culture has been demonstrated to reduce within hours of the application of hypoxia whereas, nutrient availability and concentration within the media is not rapidly affected by hypoxia (Drew and Sisworo 1979). This indicates that reduced uptake may be the primary cause of reduced nutrient status in waterlogged plants. However, in a number of plant species, the provision of additional nutrients to waterlogged plants has been reported to prevent the development of foliar nutrient deficiency, despite the reduction in root uptake (Schmull and Thomas 2000, Singh et al. 2002).

The amelioration of waterlogging-induced nutrient deficiency through the application of fertilisers has been reported in many species. This effect was first reported in barley and rice (Arnon 1937). Since then there have been a large number of studies which have reported a positive effect of fertilisation on the health, survival and growth of a number of species, under waterlogged conditions (Wetson et al. 1976, Drew et al. 1979). Different types of fertiliser and methods of application under waterlogged conditions have been investigated on a range of plant species. In rice, slow release polymer-coated nitrogen fertilisers, containing 40 % nitrogen, applied basally to the soil prior to flooding was reported to be the

most effective method for improving grain yield and recovery under waterlogged conditions (Fan et al. 1997, Carreres et al. 2003).

Singh et al. (2002) reported that nitrogen fertiliser applied to waterlogged sorghum as soluble urea in the irrigation water, significantly increased plant nutrition and yield. Applications of nitrogen fertiliser applied as a top-dressing was reported to increase the shoot growth and yield of waterlogged wheat (Swarup and Sharma 1993). Nitrogen applied as a foliar spray to winter rape seedlings was reported to alleviate plant damage caused by waterlogging (Zhou et al. 1997). Phosphorus fertiliser application during a waterlogging treatment was shown to increase the growth of waterlogged loblolly pine (Hook et al. 1983).

The application of fertiliser after a waterlogging event (remedial fertilisation) is another potential method for ameliorating the foliar deficiency resulting from waterlogging. The increased rate of nutrient uptake that occurs when waterlogged plants are drained (Lizaso et al. 2001) may increase the effectiveness of fertilisation applied after exposure to waterlogging. This method may also be more practical in the field, due to the difficulties in predicting the occurrence of a waterlogging event and the problems of avoiding run-off from fertilised areas.

The capacity of a waterlogged and anaerobic soil to retain additional nutrients is affected by (i) soil chemical properties including pH, Eh, cation exchange capacity, organic matter content, concentration of iron and aluminium oxides and clay content; (ii) size of microbial populations and (iii) the presence of other ions capable of transforming or precipitating elements into non-available forms (Phillips 2001). This can be a common problem, due to the complex number of antagonistic and synergistic interactions between the concentrations of macro- and micro-nutrients (McLaren and Cameron 1996). After fertilisation, key elements such as phosphorus can be “fixed” by iron or aluminium complexes in the soil, resulting in their reduced availability to plants, regardless of the application rate (McLaren and Cameron 1996). An example of this process is the precipitation of highly reactive iron hydrous oxides under anaerobic conditions, which affects the sorption of phosphorus and its availability to plant roots (Phillips 1998).

The effectiveness of each application method is dependent on species, soil type and period of exposure to waterlogging. In this experiment, the relationship between foliar nutrient status and application of soil-based and foliar fertiliser additions either prior to, during or following waterlogging was investigated in waterlogged *E. globulus* and *E. nitens* seedlings.

Hypotheses tested:

1. Significant reductions in foliar nutrient status in *E. globulus* and *E. nitens* seedlings will develop under waterlogged conditions.
2. The application of fertiliser will ameliorate foliar nutrient deficiency and reduce physiological symptoms of waterlogging.
3. The timing and mode of fertiliser application will affect the degree of waterlogging damage.

4.2 Materials and methods

4.2.1 Experimental design

Seedling stock

Hardened *Eucalyptus globulus* and *E. nitens* seedlings, approximately 20 cm in height, were supplied by Woodlea Nursery (Scottsdale, Tasmania) in February 2004.

Potting conditions

The potting soil used was a native soil mix (4:1, pinebark:coarse sand), supplied by Horticultural Supplies (Hobart, Tasmania). Seedlings were potted into 4.5 L pots, 200 mm in diameter. The base of each pot was covered by 50 mm of gravel to facilitate drainage. Pots were then filled with the native soil mix to within 50 mm of the top of the pot. Seedlings were staked and watered immediately after planting and daily thereafter. Pots were positioned in a randomised block design in the glasshouse, and given an establishment period of 14 weeks.

For the first four weeks, all pots were fertilised weekly with Aquasol® (Hortico Australia Pty. Ltd, Laverton North, Victoria Australia., N:P:K 23:4:18) at a rate of 1 g L⁻¹ and Iron Chelate (Kendon Chemical and Manufacturing Co. Pty. Ltd., Thornbury, Victoria Australia) at a concentration of 0.05 g L⁻¹ to stimulate growth.

Treatments

Forty-nine pots of each species were selected for experimentation based on similarity in size and condition, and divided equally into one of the following seven treatments;

1. Drained and non-fertilised (D/NF)
2. Waterlogged and non-fertilised (WL/NF)
3. Drained and fertilised (D/F)
4. Waterlogged and fertilised (WL/F)
5. Waterlogged and secondary soil fertiliser application after three weeks of waterlogging (WL/Sec)

6. Waterlogged and secondary foliar fertiliser application after three weeks of waterlogging (WL/Fol)
7. Waterlogged and remedial soil fertiliser application at the end of the waterlogging treatment (WL/Rem)

Waterlogging was applied by submerging the pots in 10 L buckets filled with tap water. Water level was maintained at the soil surface for the duration of the 10 week treatment. A recovery period (freely drained conditions) of four weeks was then applied.

Non-fertilised treatments only received the four weeks of Aquasol® fertiliser applied to all seedlings when seedlings were first established in the glasshouse. Fertilised treatments received the four week Aquasol® application and a sub-surface application of Osmocote® (14.6 : 6.1 : 11.6, N : P : K) applied one week prior to waterlogging as four 30 g plugs inserted 30 mm below the soil surface. This prevented algal growth on the surface of waterlogged pots.

Secondary soil fertilisation was applied after three weeks of waterlogging. It was applied as a plug of Osmocote®, in the same manner as described above.

Foliar application of fertiliser was achieved by applying of 50 mL of Aquasol® to the adaxial leaf surface of all fully expanded leaves per seedling. Application was repeated on three successive days after three weeks of waterlogging.

Remedial fertilisation was applied 24 hours after drainage of the waterlogging treatment as a subsurface plug of Osmocote®, in the same manner as described above.

4.2.2 Measurements

Growth

Seedling height and survival was measured on a subset of three plants from both species and all treatments fortnightly during the waterlogging and recovery periods.

Plant biomass harvests were conducted on three randomly selected seedlings (i) at the end of the 10 week waterlogging period; and (ii) at the end of the four week recovery period that followed the waterlogging treatment. Seedling height, stem diameter, stele diameter, leaf number and dry biomass of root, stem and leaf were recorded.

Physiology

Physiological measurements were conducted on the subset of plants used for ongoing height measurements. Physiological measurements were made on three seedlings from each species in each treatment at the following times: on the day prior to the imposition of waterlogging; after three weeks of waterlogging; after six weeks of waterlogging; at the end of the 10 week waterlogging treatment; two weeks after waterlogging stopped, and; at the completion of the four week recovery period.

Leaf water potential

Predawn leaf water potential was recorded using a pressure chamber (PMS Instrument Co., Corvallis, Oregon) (Scholander et al. 1965). A mature leaf from the lowest third of the crown was excised and enclosed within the chamber and the balance pressure obtained within one minute.

Photochemical efficiency

Predawn F_v/F_m measurements were made on three of the most recently expanded leaves using the portable PAM-2000 fluorometer (Heinz Walz GmbH, Elfelrich, Germany) and 2030-B leaf-clip.

Foliar nutrition

Dried leaf material collected from harvested seedlings was used for foliar nutrient analysis. Total foliage was used due to the size of seedlings, and to measure total plant nutrient status. Macronutrient (nitrogen, phosphorus, potassium, magnesium and calcium) concentrations were measured for both species from samples collected at harvest 1. Waterlogged/remedial fertilised samples were collected at harvest 2 (after four week recovery) and compared to waterlogged/non-fertilised samples at harvest 2. Macronutrient analysis was conducted using the sulphuric

acid/hydrogen peroxide digest method (Lowther 1980). Micronutrient (copper, zinc, iron and manganese) concentrations were measured in *E. globulus* samples only collected at the same times as above. The Nitric Acid digest method was used to analyse micronutrients, and is the method used by the State Government Department of the Environment and Land Management (DELM).

Three blanks and three reference standards were included in each analysis. The reference standards used for quantification were Euc123, Pine 43 and Pine 9 and it was ensured that the results of these reference standards were within 5% of the known quantity of analyte.

4.2.3 Statistical analyses

A three-way analysis of variance was performed using the proc and GLM procedures of SAS (SAS Institute, 1996) to test the effects of waterlogging, fertilisation and species on growth measurements at the two harvests.

Physiological measurements were analysed similarly. All variables were checked for normality and transformed if required.

4.3 Results

4.3.1 Growth

Height

Prior to the application of waterlogging, *E. nitens* seedlings were significantly smaller (35-62 cm) than *E. globulus* (66-87 cm) across all treatment groups (Figure 4.1).

After 10 weeks of waterlogging, there was no significant difference in the height of *E. globulus* between waterlogged treatments. At the end of the recovery period, drained/fertilised seedlings were significantly taller than all other treatments. In *E. nitens*, the treatment with the largest increase in height was drained/fertilised seedlings (Figure 4.1).

Relative growth rate (RGR)

Fertilised drained and fertilised waterlogged *E. globulus* seedlings exhibited higher RGR than other treatments during the waterlogging and recovery periods (Table 4.1). Secondary and remedial fertiliser treatments showed increased RGR during the recovery period. A similar pattern can be seen for *E. nitens*, except that the effect of fertilising during waterlogging and following waterlogging (remedial) was less obvious. Foliar fertilisation had no positive effect on seedling RGR for either species.

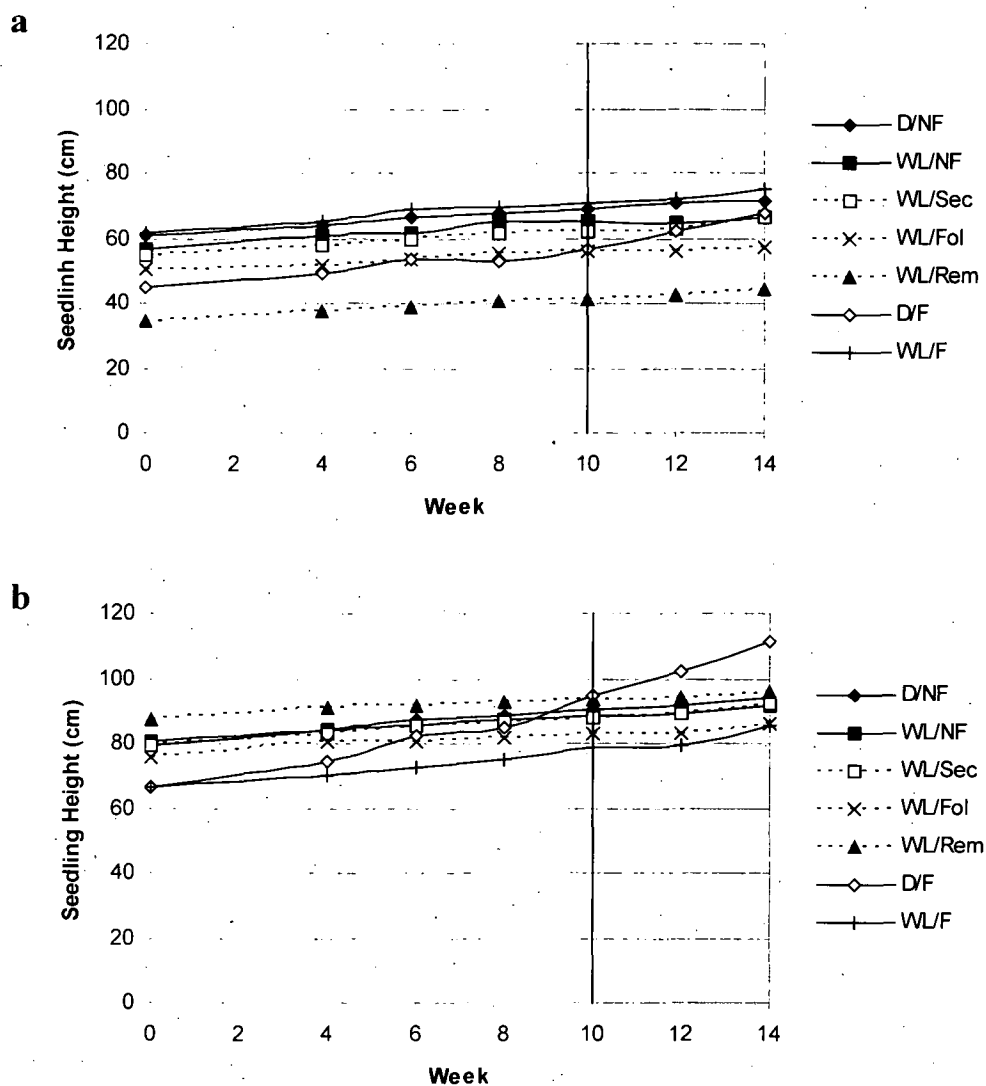


Figure 4.1 Mean seedling height (cm) of *E. nitens* (a) and *E. globulus* (b) over the experimental period. The conclusion of waterlogging was at week 10 (represented by the vertical line). Largest standard error = 9.2 (*E. nitens*) and 13.8 (*E. globulus*).

D = drained, WL = waterlogged, NF = non-fertilised, Sec = secondary fertilisation, Fol = foliar fertilisation, Rem = remedial fertilisation and F = fertilised.

Table 4.1 Relative growth rate ($\text{cm cm}^{-1} \text{ week}^{-1}$) of *E. globulus* and *E. nitens* seedlings during the 10 week waterlogging treatment, and subsequent 4 weeks recovery period.

Treatment	RGR ($\text{cm cm}^{-1} \text{ week}^{-1}$) during 10 weeks waterlogging	RGR ($\text{cm cm}^{-1} \text{ week}^{-1}$) during 4 weeks recovery
<i>E. globulus</i>		
Drained / Non-fertilised	0.0129	0.0084
Drained / Fertilised	0.0362	0.0422
Waterlogged / Non-fertilised	0.0102	0.0087
Waterlogged/ Fertilised	0.0172	0.0214
Waterlogged / 2 Fert	0.0107	0.0128
Waterlogged / Foliar Fert	0.0093	0.0082
Waterlogged / Remedial Fert	0.0065	0.0072
<i>E. nitens</i>		
Drained / Non-fertilised	0.0125	0.0075
Drained / Fertilised	0.0218	0.0456
Waterlogged / Non-fertilised	0.0137	0.0065
Waterlogged / Fertilised	0.0136	0.0153
Waterlogged / 2 Fert	0.0117	0.0172
Waterlogged / Foliar Fert	0.0093	0.004
Waterlogged / Remedial Fert	0.0176	0.0176

4.3.2 Harvest 1 (after 10 weeks of waterlogging)

Stem diameter

At the end of the 10 week waterlogging treatment (harvest 1) *E. globulus* had greater stem diameter than *E. nitens* across all treatments (Figure 4.2), except drained/non-fertilised. This was probably due to the larger initial size of the *E. globulus* seedlings. Waterlogged *E. globulus* seedlings had increased stem diameter (Figure 4.2) due to stem hypertrophy.

Aerenchymatous root development

Eucalyptus globulus had significantly greater aerenchymatous root production than *E. nitens* across most treatments (Figure 4.2). Soil-based fertilisation was the most effective in increasing the development of aerenchymatous roots in *E. globulus*.

Total plant dry weight

Eucalyptus globulus had significantly greater mean seedling dry weight than *E. nitens* ($P < 0.0001$, Figure 4.2). The significant interaction between species and fertilisation (Table 4.2) indicates that *E. globulus* a greater increase in plant dry weight than *E. nitens* in response to soil-based fertilised treatments.

Root:shoot ratio

Eucalyptus nitens had a significantly greater root:shoot ratio than *E. globulus* after 10 weeks of waterlogging ($P = 0.0003$) (Figure 4.2). There was also a significant interactive effect of waterlogging and fertilisation on root:shoot ratio (Table 4.2). Fertilisation applied to drained seedlings resulted in lower root:shoot ratio, compared to waterlogged seedlings which had higher root:shoot ratios under fertilised conditions (Figure 4.2). There was no effect of the type of fertiliser application on root:shoot ratio, and there was a similar response to all application modes in both species.

Table 4.2 Significant effects ($P < 0.05$) of species, waterlogging and fertilisation on seedling height, stem diameter, leaf number, biomass components, root length and the development of aerenchymatous roots at harvest 1 (after 10 weeks of waterlogging).

Variable	Species	Water	Fert	Spp* water	Spp*fert	Water* fert	Spp* water* fert
Height	<0.0001	0.0092			0.0316		
Stem Diameter	0.0015	0.0051		0.0488	0.0103		
Stele diameter	0.0042						
Leaf number	<0.0001	0.0147					
Leaf Fwgt	<0.0001	0.0363			0.0161		
Stem Fwgt	0.0003				0.0067		
Leaf Dwgt	<0.0001				0.0246		
Stem Dwgt	0.0002				0.0086		
Root Fwgt					0.0168		
Root Dwgt	0.036						
Primary root length	0.0024						
Aer Fwgt	0.0006		0.0174	0.0174	0.0151		
Total Dwgt	<0.0001				0.012		
R:shoot ratio	0.0003					0.0064	

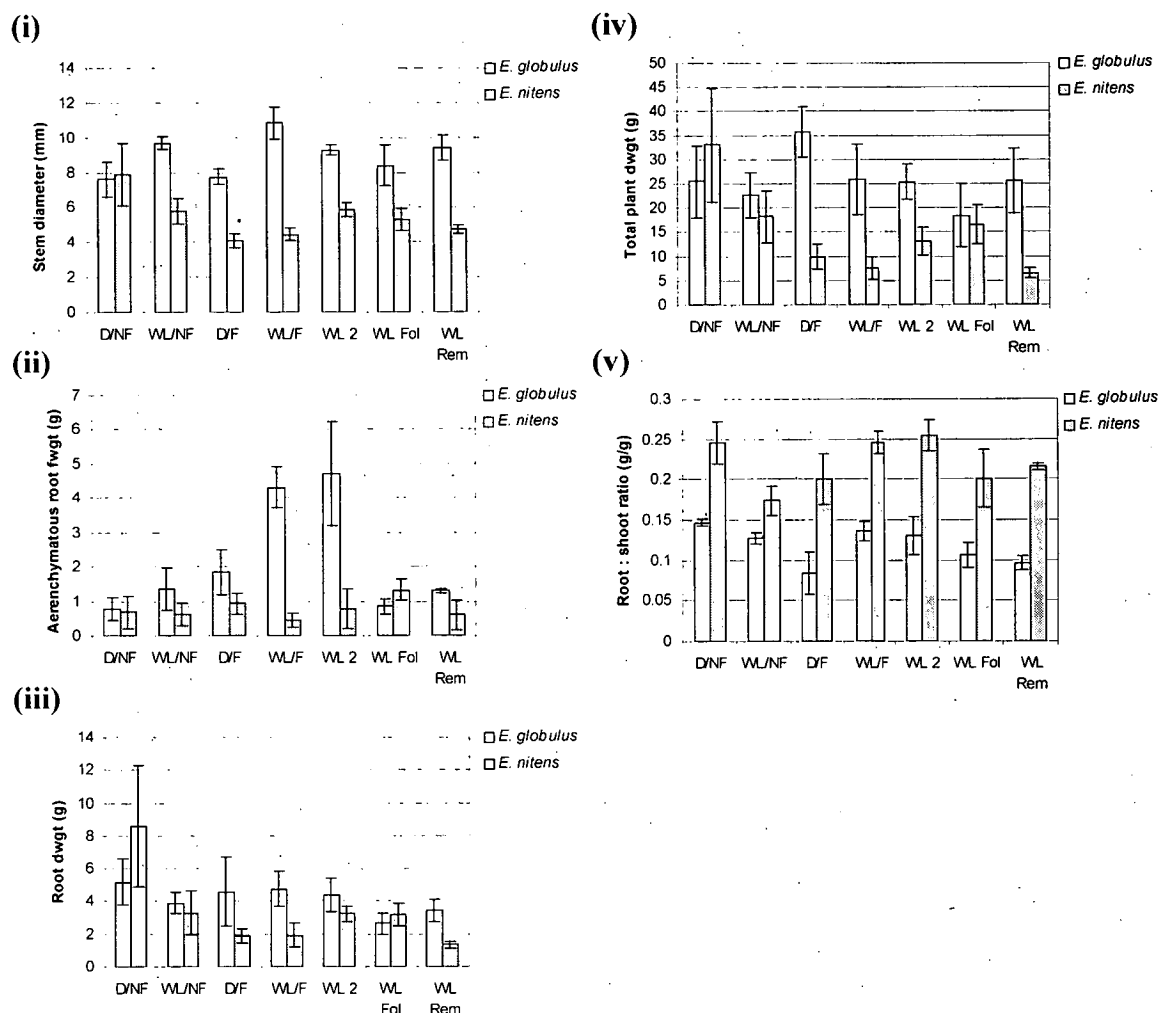


Figure 4.2 Mean seedling stem diameter (i), development of aerenchymatous roots (ii), root dry weight (iii), total plant dry weight (iv) and root:shoot ratio (v) of *E. globulus* and *E. nitens* after 10 weeks of waterlogging (harvest 1).

D = drained, WL = waterlogged, NF = non-fertilised, F = fertilised, 2 = secondary soil fertilisation, Fol = foliar fertilisation, Rem = remedial fertilisation. Bars represent standard error.

4.3.3 Harvest 2 (after four weeks of recovery)

Stem diameter

Eucalyptus globulus had significantly greater stem diameter than *E. nitens* ($P = 0.008$, Figure 4.3) after the four week recovery period, similarly as at the end of the waterlogging treatment, but differences were much reduced. The average seedling stem diameter over the four week recovery period had increased, particularly in *E. nitens*.

Aerenchymatous root development

There was a significant increase in the fresh weight of aerenchymatous roots of *E. globulus* associated with soil based fertilisation prior to or during waterlogging (Figure 4.2). This trend was not evident in *E. nitens*.

The fresh weight of aerenchymatous roots of *E. globulus* had significantly increased in the drained/non-fertilised, waterlogged/non-fertilised and waterlogged/remedial fertilised seedlings after four weeks recovery from waterlogging. All treatments of *E. nitens* had an increase in the fresh weight of aerenchymatous roots after the recovery period. Fertilisation had the effect of decreasing the aerenchyma development in drained treatments, and increasing it under waterlogged conditions.

In *E. nitens*, the soil based application of fertiliser prior to waterlogging increased aerenchymatous root development.

Total dry weight

Eucalyptus globulus had a significantly higher mean plant dry weight than *E. nitens* across most treatments after four weeks recovery (Table 4.3, Figure 4.3). There was no significant effect of fertiliser treatment on the total biomass of waterlogged *E. globulus* seedlings. *Eucalyptus nitens* seedlings exhibited a reduction in total plant biomass associated with waterlogging.

Root dry weight

Root dry weight of seedlings increased substantially between the end of the waterlogging period (Figure 4.2) and the end of the recovery period (Figure 4.3) in all treatments, except for waterlogged *E. nitens* receiving foliar fertiliser. *Eucalyptus globulus* had significantly greater root dry weight than *E. nitens* after the four week recovery treatment ($P = 0.0002$, Table 4.3, Figure 4.3). The significant interactive effect of fertiliser and species (Table 4.3) is indicative of the different response of the two species to fertilisation. *Eucalyptus globulus* had greater root development than *E. nitens* in fertilised treatments.

Root:shoot ratio

There was no effect of species on root:shoot ratio after four weeks recovery (Figure 4.3). Fertiliser applied prior to waterlogging decreased the root:shoot ratio, whereas, secondary (both soil-based and foliar applications) and remedial fertilisation increased the root:shoot ratio in both species under waterlogged conditions (Figure 4.3).

Table 4.3 Significant effects ($P < 0.05$) of species, waterlogging and fertilisation on seedling height, stem diameter, leaf number, biomass components (fresh and dry weights), root length and the development of aerenchymatous roots at harvest 2 (after four weeks of recovery).

Variable	Species	Water	Fert	Spp* water	Spp*fert	Water* fert	Spp* water* fert
Height	<0.0001						0.0138
Stem diameter	0.008		0.0161				
Stele diameter	0.0004	0.0505	0.0410				
Leaf number	<0.0001		0.0007				
Leaf Fwgt	<0.0001		0.0018				
Stem Fwgt	<0.0001		0.0108				
Leaf Dwgt	0.0001		0.0082				
Stem Dwgt	<0.0001		0.0262				
Root Fwgt	0.0014		0.0061				
Root Dwgt	0.0002		0.0042		0.0432		
Secondary root length		0.0065					
Aer Fwgt		0.0159	0.0196				
Total Dwgt	<0.0001		0.0021				
R:shoot ratio							

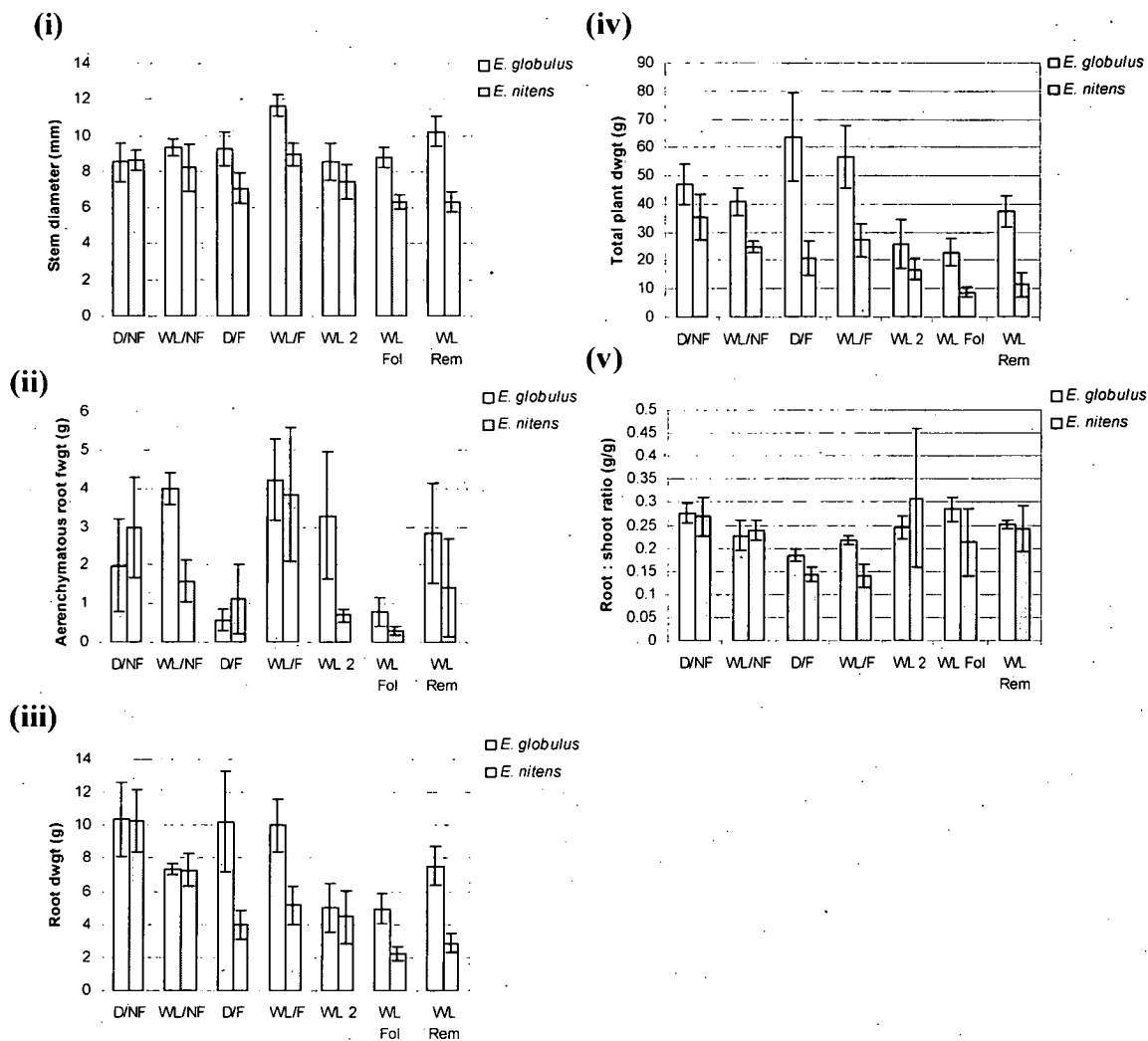


Figure 4.3 Mean seedling stem diameter (i), development of aerenchymatous roots (ii), root fry weight (iii), total plant dry weight (iv) and root:shoot ratio (v) of *E. globulus* and *E. nitens* after four weeks of recovery (harvest 2). D = drained, WL = waterlogged, NF = non-fertilised, F = fertilised, 2 = secondary fertilisation, Fol = foliar fertilisation, Rem = remedial fertilisation. Bars represent standard error.

4.3.4 Physiology

Leaf water potential

There was no significant effect of waterlogging on the predawn leaf water potential (see Appendix 5.1 – 5.5) of either species. The average predawn water potential during waterlogging was;

E. globulus Drained = -0.32 MPa, waterlogged = -0.35 MPa

E. nitens Drained = -0.35 MPa, waterlogged = -0.35 MPa

Photochemical efficiency

Prior to the application of waterlogging, the predawn F_v/F_m of *E. globulus* was lower than *E. nitens* (Figure 4.4). After three weeks of waterlogging, the only depression in F_v/F_m observed was in drained/non-fertilised *E. globulus* seedlings. In all other treatments the F_v/F_m of *E. globulus* seedlings was lower than *E. nitens* (Figure 4.5). However, no differences were ecologically significant (i.e. no value < 0.6 indicating photoinhibition).

After six weeks of waterlogging, all treatments for both species had an F_v/F_m of > 0.8 (Figure 4.6) except the waterlogged/remedial *E. globulus* seedlings (F_v/F_m < 0.78).

At the conclusion of 10 weeks of waterlogging (Figure 4.7), there were significant reductions ($P < 0.01$) in the F_v/F_m of non-fertilised, secondarily fertilised and remedial fertilised waterlogged *E. globulus* seedlings. The F_v/F_m of *E. nitens* foliar and remedial fertilised seedlings was also significantly reduced after 10 weeks of waterlogging ($P < 0.001$).

The F_v/F_m of *E. globulus* seedlings had recovered in all treatments, except waterlogged/foliar fertilisation at the end of the 4 week recovery period (Figure 4.8). *Eucalyptus globulus* seedlings had lower F_v/F_m ratios than *E. nitens* in almost all treatments. The F_v/F_m of waterlogged/remedial *E. nitens* seedlings had recovered to > 0.8 after 4 weeks of recovery.

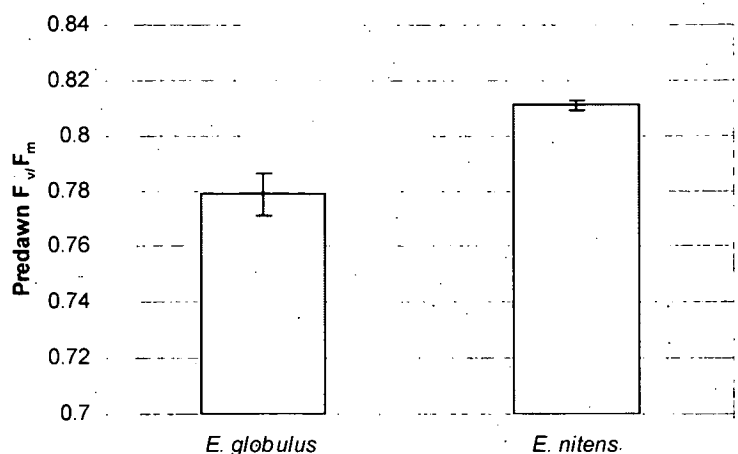


Figure 4.4 Predawn F_v/F_m ratio of *E. globulus* and *E. nitens* prior to waterlogging (May 2003). Bars represent standard error.

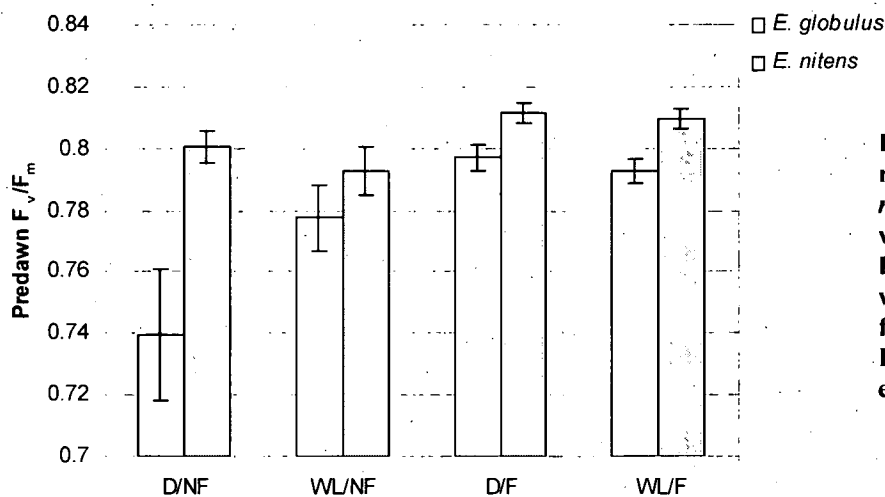


Figure 4.5 Predawn F_v/F_m ratio of *E. globulus* and *E. nitens* after three weeks waterlogging (8 June 2003). D = drained, WL = waterlogged, NF = non-fertilised and F = fertilised. Bars represent standard error.

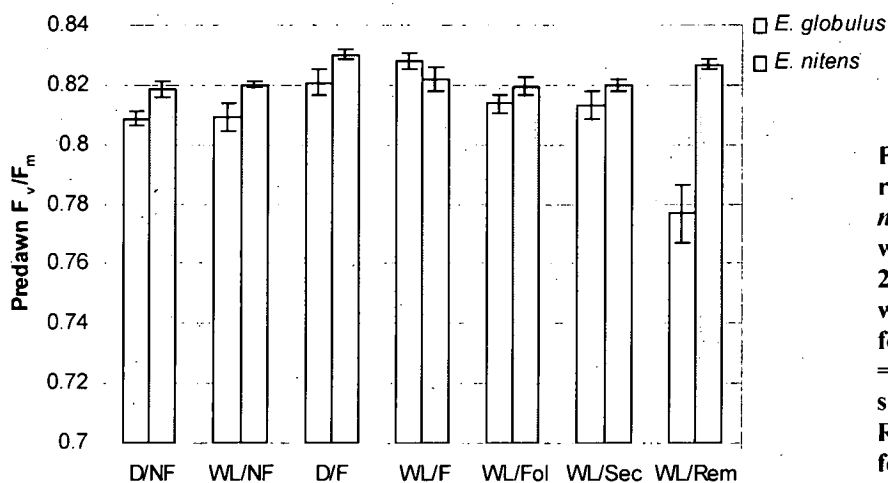


Figure 4.6 Predawn F_v/F_m ratio of *E. globulus* and *E. nitens* after six weeks waterlogging (29 June 2003). D=drained, WL = waterlogged, NF = non-fertilised, F = fertilised, Fol = foliar fertilisation, Sec = secondary fertilisation and Rem = remedial fertilisation. Bars represent standard error.

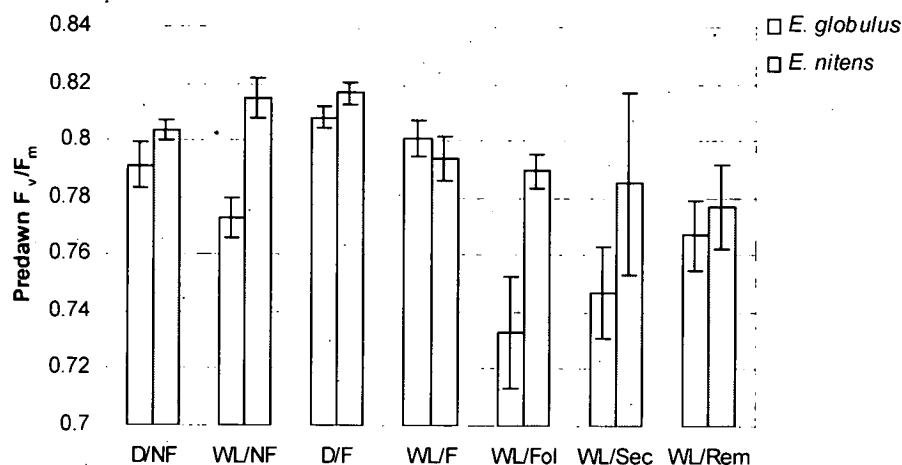


Figure 4.7 Predawn F_v/F_m ratio of *E. globulus* and *E. nitens* after 10 weeks of waterlogging (27 July 2003). D = drained, WL = waterlogged, NF = non-fertilised, F = fertilised, Fol = foliar fertilisation, Sec = secondary fertilisation and Rem = remedial fertilisation. Bars represent standard error.

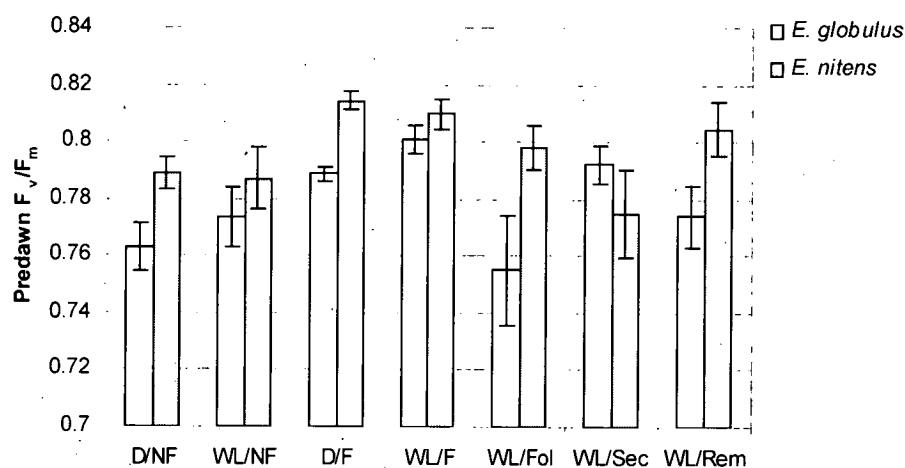


Figure 4.8 Predawn F_v/F_m ratio of *E. globulus* and *E. nitens* after 4 weeks of recovery (24 August 2003). D = drained, WL = waterlogged, NF = non-fertilised, F = fertilised, Fol = foliar fertilisation, Sec = secondary fertilisation and Rem = remedial fertilisation. Bars represent standard error.

4.3.5 Foliar nutrition

Macronutrient concentration

Pre-waterlogging fertilisation significantly increased ($P < 0.05$) the foliar concentrations of nitrogen and phosphorus in drained *E. globulus*, with no effect on potassium, magnesium or calcium (Figure 4.9). *Eucalyptus nitens* seedlings fertilised prior to waterlogging had higher nitrogen and phosphorus, and reduced magnesium and calcium concentrations (Figure 4.10).

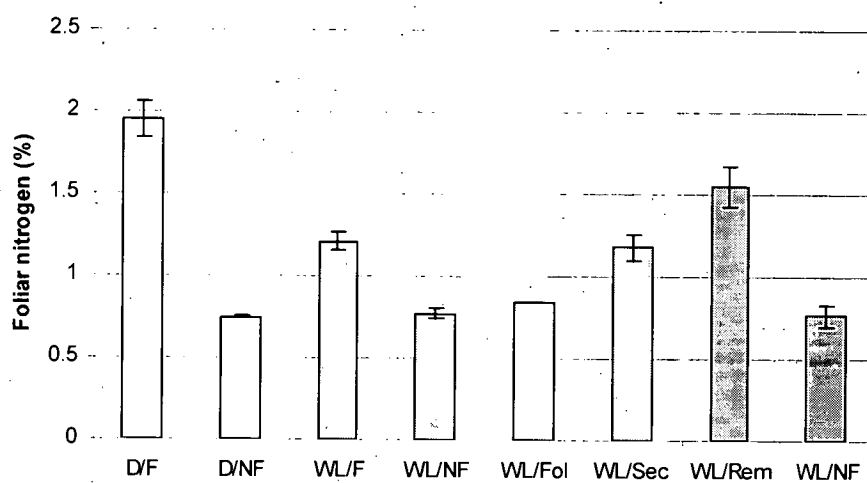
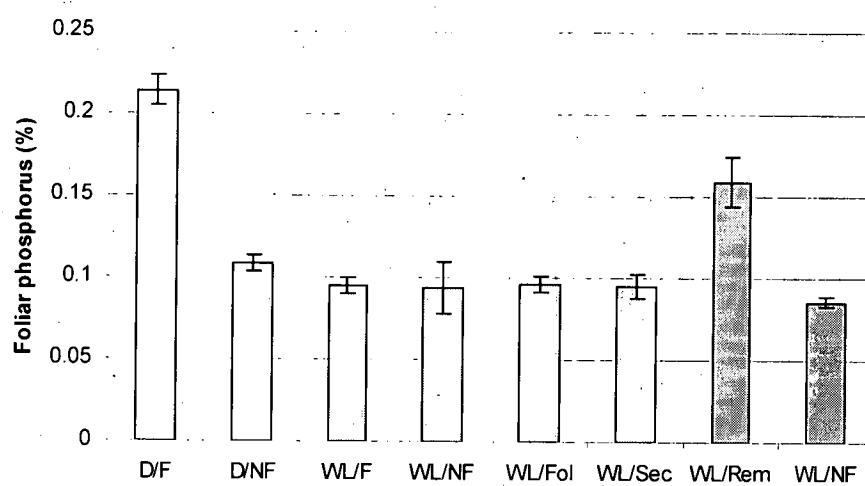
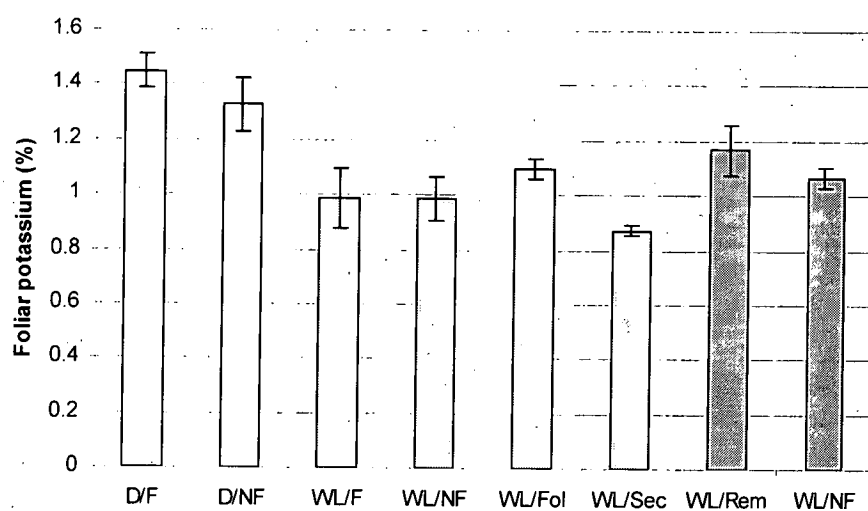
Waterlogging significantly reduced the concentration of nitrogen, phosphorus, potassium, magnesium and calcium in fertilised treatments for both species ($P < 0.0001$, Table 4.4, Figure 4.9). Soil-based fertiliser application (pre-waterlogging, secondary and remedial) had a positive effect on the foliar nitrogen and phosphorus concentrations under drained and waterlogged conditions.

Foliar fertiliser was ineffective in increasing foliar nutrient status under waterlogged conditions.

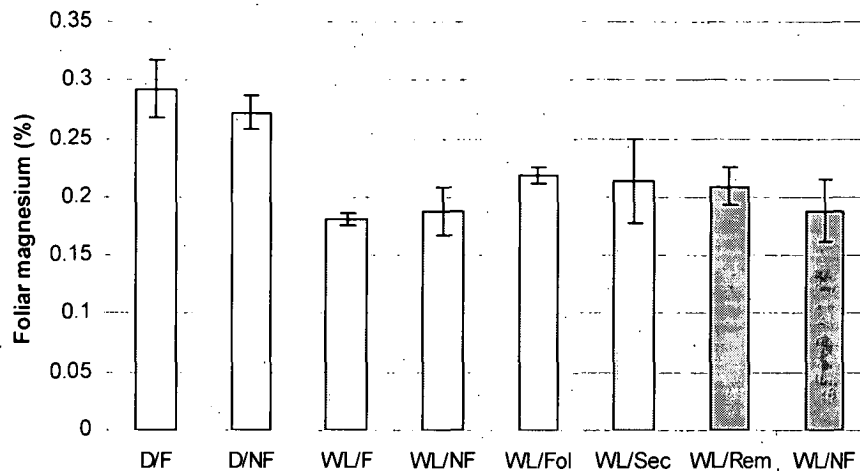
There was an increase in foliar concentration of magnesium and calcium in waterlogged/non-fertilised seedlings during the four week recovery period.

Table 4.4 Significant effects (P values < 0.05) of species, fertilisation and waterlogging on the foliar concentrations of macronutrients in *E. globulus* and *E. nitens* seedlings at harvest 1 (after 10 weeks of waterlogging).

Element	Species	Fertilisation	Water	Fert * Water
Nitrogen	0.0046	<0.0001	<0.0001	0.0001
Phosphorus	<0.0001	<0.0001	0.0001	0.0006
Potassium	0.0029		<0.0001	
Calcium	<0.0001	0.0003	<0.0001	
Magnesium	<0.0001		<0.0001	

a**b****c**

d



e

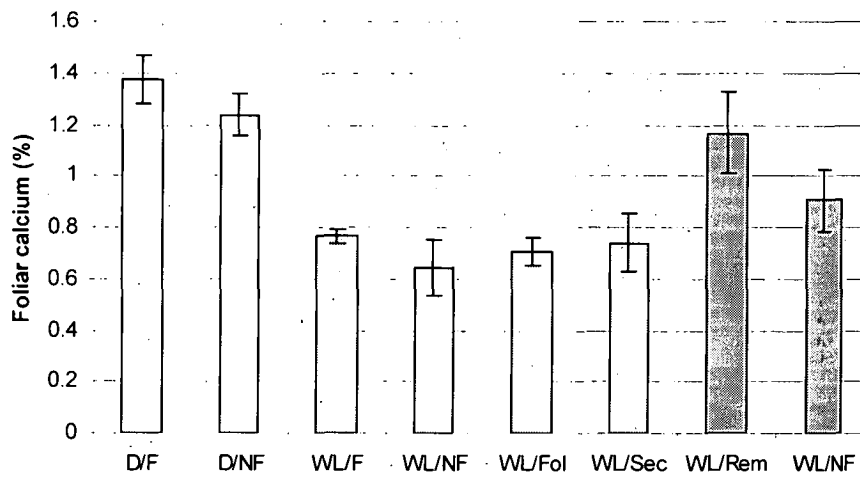
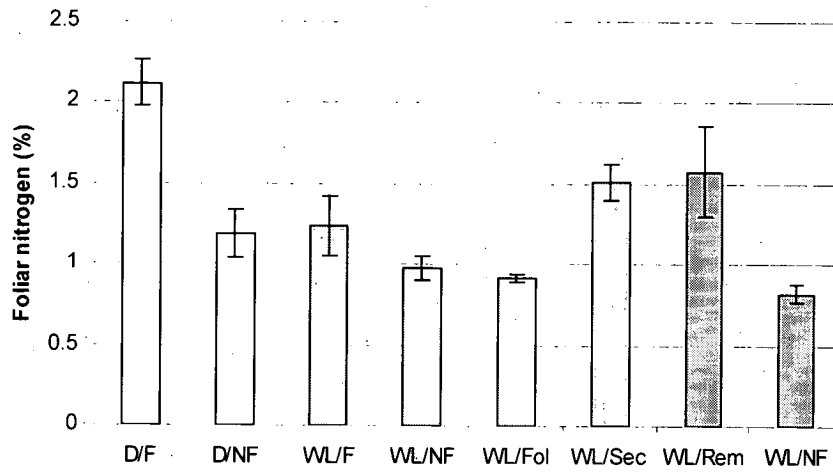
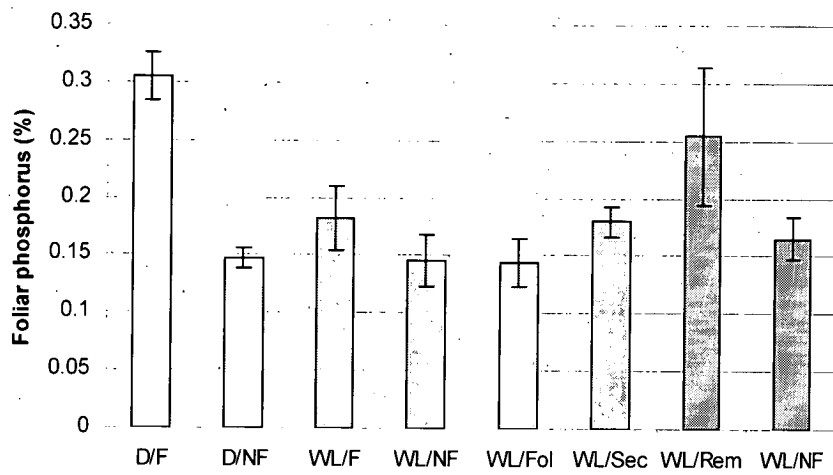
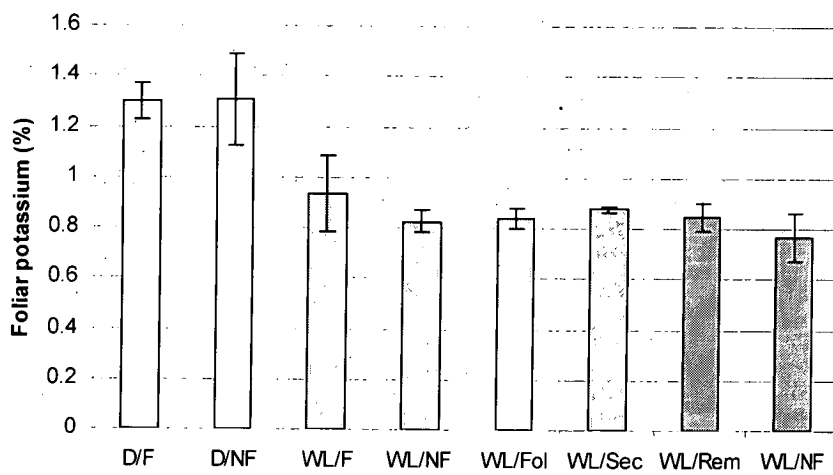


Figure 4.9 Foliar concentration (%) of nitrogen (a), phosphorus (b), potassium (c), magnesium (d) and calcium (e) in *E. globulus* seedlings after 10 weeks of waterlogging (harvest 1). D = drained, WL= waterlogged, F = fertilised, NF = non-fertilised, Fol = foliar fertilisation, Sec = secondary fertilisation, Rem = remedial fertilisation. Note tissue used to analyse WL/Rem and WL/Non (dark) was from harvest 2. Bars represent standard error.

a**b****c**

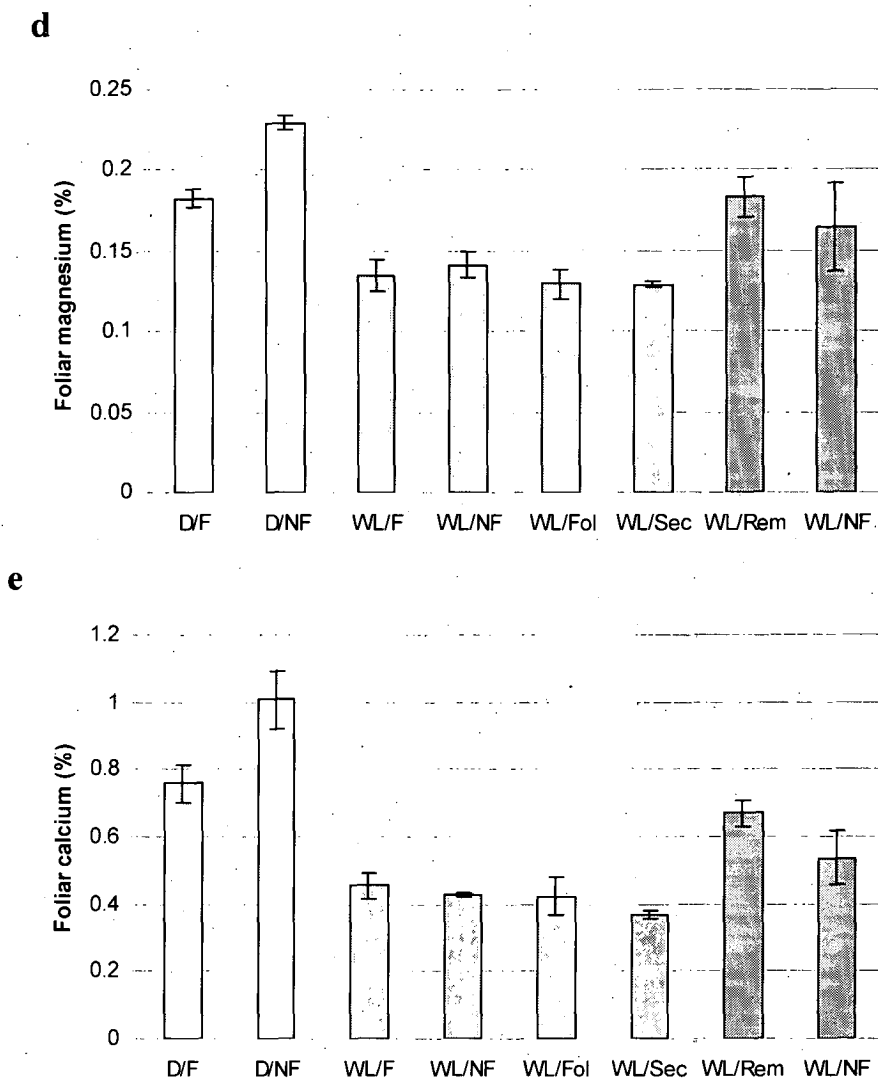


Figure 4.10 Foliar concentrations (%) of nitrogen, phosphorus, potassium, magnesium and calcium in *E. nitens* seedlings after 10 weeks of waterlogging (harvest 1). D = drained, WL = waterlogged, F = fertilised, NF = non-fertilised, Fol = foliar fertilisation, Sec = secondary fertilisation, Rem = remedial fertilisation. Note tissue used to analyse WL/Rem and WL/Non (dark) was from harvest 2. Bars represent standard error.

Micronutrient concentration

Fertilisation and waterlogging had a significant effect on copper, manganese and zinc concentrations (Table 4.5). The only significant interactive effect of these two factors was on foliar iron concentration. Fertilisation of drained seedlings increased foliar iron concentration, compared to a decrease in iron concentration of fertilised seedlings under waterlogged treatments (Figure 4.11).

Pre-waterlogging fertilisation had a positive effect on the foliar manganese concentration in *E. globulus*. There was no significant effect of fertilisation or waterlogging on the foliar iron concentration in *E. globulus*.

Remedial fertilisation significantly increased foliar copper iron, manganese and zinc concentrations after 4 weeks of drainage in waterlogged *E. globulus*.

Table 4.5 Significant effects (P values < 0.05) of fertilisation and waterlogging on the foliar concentrations of micronutrients in *E. globulus* seedlings at harvest 1 (after 10 weeks of waterlogging).

Element	Fertilisation	Water	Fert * Water
Copper	0.0004	0.0424	
Iron			0.0285
Manganese	0.006	0.001	
Zinc	0.0089	0.0481	

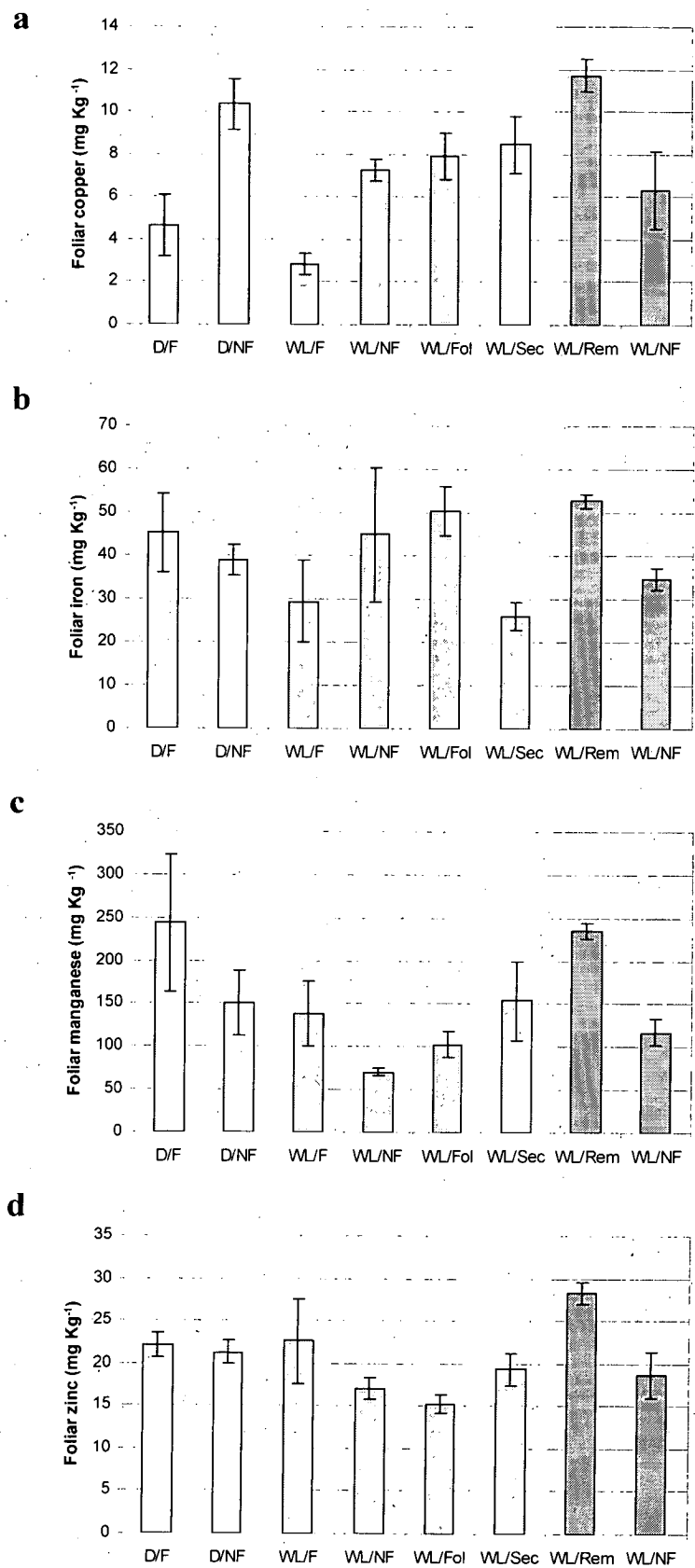


Figure 4.11 Foliar concentrations (mg kg⁻¹) of copper (a), iron (b), manganese (c) and zinc (d) in *E. globulus* seedlings at harvest 1. D = drained, WL= waterlogged, F = fertilised, NF = non-fertilised, Fol = foliar fertilisation, Sec = secondary fertilisation, Rem = remedial fertilisation. Note tissue used to analyse WL/Rem and WL/Non (dark) was from harvest 2. Bars represent standard error.

4.4 Discussion

In the current study, the two eucalypt species differed in their response to fertiliser application during the waterlogging period, with *E. nitens* indicating no increase in RGR associated with fertilisation until after the waterlogging condition was removed. In comparison, *E. globulus* responded positively to the application of fertiliser, with a significant increase in RGR. This is consistent with the results of the previous chapter, indicating that *E. globulus* is relatively more sensitive to fertiliser, and *E. nitens* to waterlogging. The inherently higher nutrient status of *E. globulus* compared to *E. nitens* (Close and Beadle 2004) indicates this species has a higher requirement for nutrients, and the positive effect of fertiliser on the growth of *E. globulus* in this experiment maybe a factor of the species' high requirement for nutrients. The ability to effectively take up and accumulate nutrients during waterlogging is clearly demonstrated by the increased growth of *E. globulus* seedlings under fertilised conditions.

The development of aerenchymatous tissue is the primary morphological adaptation of waterlogged plants. The formation of cortical air spaces in the stem and root enables the diffusion of oxygen from aerial parts of the plant to the oxygen-deficient root zone (Evans 2003). The capacity of eucalypt species to develop aerenchyma is a measure of the relative tolerance to waterlogging (Clemens and Pearson 1977, Connell 1998). In *E. camaldulensis*, the development of adventitious roots has been associated with recovery in plant water status and water use efficiency under waterlogged conditions (Akilan et al. 1997).

In the present study, *E. globulus* displayed a greater level of aerenchymatous root development than *E. nitens*. The relatively short application of 10 weeks of waterlogging induced the production of aerenchymatous roots in *E. globulus*, particularly in conjunction with soil-based fertiliser applications. There was a more than 200 % increase in the fresh weight of aerenchymatous root tissue in waterlogged and fertilised *E. globulus* seedlings after 10 weeks. In *E. nitens* there was no increase in the development of aerenchymatous roots of waterlogged

seedlings compared to the drained treatment, and fertiliser had little effect on the development of aerenchyma during the waterlogging treatment.

Waterlogging results in root death and reduction in the root:shoot ratio of plants (Brisson et al. 2002). *Eucalyptus nitens* had a significantly higher root:shoot ratio than *E. globulus* across all treatments after 10 weeks of waterlogging, consistent with reports that *E. nitens* produces a larger root system than *E. globulus* (Moroni et al. 2003). Therefore, despite the high degree of aerenchymatous root development in *E. globulus*, the species had a relatively smaller root system compared to *E. nitens*, indicating that a high percentage of the root system was composed of aerenchymatous roots, and that *E. nitens* retained a larger functional primary root system during the waterlogging treatment.

The development of aerenchymatous tissue in *E. globulus* and *E. nitens* during the freely-drained recovery period supports the hypothesis that aerenchymatous roots are produced in response to other environmental conditions, including nutrient deficiency (Evans 2003). It has been demonstrated that aerenchymatous roots are equally efficient as non-aerenchymatous roots in nutrient uptake (Lizaso et al. 2001). Therefore, the high degree of aerenchymatous root development following waterlogging may assist in the increased uptake of nutrients often observed in post-waterlogged plants (Lizaso et al. 2001).

The development of foliar deficiency under waterlogged conditions has been reported in a number of species, including oak (Angelov et al. 1996), cotton (Bange et al. 2004), soybean (Bacanamwo and Purcell 1999), grasses (Chen et al. 2005) hakea (Poot and Lambers 2003) and some eucalypt species (Van der Moezel et al. 1988 and Close and Davidson 2003). In this experiment, significant reductions in foliar nitrogen, phosphorus, potassium, magnesium and calcium in *E. globulus* and *E. nitens* developed under the 10 week waterlogging treatment. *Eucalyptus globulus* had an inherently higher nutrient status than *E. nitens*, which is consistent with other reports (Close and Beadle 2004).

The significantly higher foliar concentrations of nitrogen and phosphorus in waterlogged *E. globulus* and *E. nitens* seedlings which had received a soil based application of fertiliser before or during the waterlogging treatment supports the hypothesis that reduced uptake of nutrients under waterlogged conditions can be countered through the addition of nutrients to the soil. When additional nutrients were supplied to the soil during waterlogging, seedlings were capable of accumulating these nutrients, indicating sufficient uptake capacity of the root system. Uptake rate could then be improved through fertilisation.

A range of modes of fertiliser application have been successful in improving the growth and productivity of waterlogged crop species, including slow release, soluble and foliar fertilisers (Fan et al. 1997, Singh et al. 2002 and Zhou et al. 1997). In this experiment, soil based fertiliser application was the most effective, with an increase in the fresh weight of aerenchymatous roots when fertiliser was applied as a slow release subsurface plug. The two species responded similarly to the mode of fertiliser application, but differently to the timing fertiliser addition. *Eucalyptus globulus* responded positively to soil-based fertiliser applied prior to, during and after exposure to waterlogged conditions. In contrast, the only effective timing of soil-based fertiliser application in increasing the aerenchymatous root development of *E. nitens* was a pre-waterlogging application, even then, aerenchymatous root development was only evident upon drainage (after recovery period) in *E. nitens*. This is consistent with the growth results which indicate that *E. nitens* does not respond to fertiliser application during waterlogging. There was a general increase in the root:shoot ratio of seedlings during the recovery period, indicating the preferential root growth of both species, as a result of the increased aerenchymatous root production.

There is evidence of the ability of eucalypt species to take up and retain nitrogen and phosphorus at levels exceeding the physiological demand (Close et al. 2004, Hooda and Weston 1999). Therefore, pre-waterlogging fertilisation may increase the status of these two elements, which will be beneficial during subsequent waterlogging events. This may explain the positive response of *E. globulus* to pre-waterlogging fertilisation.

Soil based fertiliser applications were found to significantly increase the foliar nitrogen concentration of waterlogged *E. globulus* and *E. nitens* seedlings. The only effective fertiliser application for the amelioration of phosphorus and calcium deficiencies was a post-waterlogging soil based application. No positive effect was obtained by fertiliser application on potassium or magnesium concentrations. Therefore, the most effective method for ameliorating any foliar nutrient deficiencies of eucalypts in waterlogged soils is a soil-based application of slow-release fertiliser, after waterlogging.

The application of soil-based fertiliser increased foliar nutrition and was associated with improved growth and increased development of morphological adaptations in both species.

~ CHAPTER 5 ~

Hypoxic pre-conditioning of *E. globulus* and *E. nitens* seedlings prior to exposure to waterlogging**5.1 Introduction**

During a natural waterlogging event there is a slow decline in the oxygen concentration of the rooting environment, which may occur over several days (Gibbs and Greenway 2003). The rate of oxygen depletion is dependent on a number of factors, including soil temperature, respiratory demand from plants and microbes and soil organic matter content (Jackson and Drew 1984, Phillips 1999). The slow imposition of hypoxic (oxygen deficient) conditions under glasshouse conditions may be used as a pre-treatment that imitates the gradual development of waterlogged conditions typically encountered by plants in the field (Voesenek et al. 1999), and represents a potential method for pre-conditioning seedlings that are to be planted into seasonally waterlogged plantations.

The application of hypoxic preconditioning to improve waterlogging-tolerance of excised roots and plants has been investigated in a number of studies (Anderson and Pezeshki 2001, Ellis and Setter 1999, Johnson et al. 1989, 1994, Voesenek et al. 1999, Marcar 1993). Hypoxic pre-conditioning has been demonstrated to result in the greater survival and viability of maize, wheat, *Rumex*, wheat, tomato and red beet (Waters et al. 1991, Johnson et al. 1989, Zhang and Greenway 1994, Drew 1997, Germain et al. 1997, Vartepatian and Jackson 1997). Increased survival of plants exposed with hypoxic conditions has been correlated to changes in metabolic processes and up-regulation of anaerobic respiration (Vartepatian and Jackson 1997).

Ethylene production under hypoxic conditions in partially flooded systems activates hydrolytic enzymes, resulting in the degradation of cell walls and formation of aerenchyma in the cortex of root or stem (Evans 2003, Vartepatian

and Jackson 1997). In hypoxically treated plants the induction of aerenchyma is associated with acclimation to waterlogging and increased tolerance (Visser et al. 1996). Little research has been conducted on the role of aerenchyma developed during hypoxic pre-treatment in the acclimation of plants to waterlogged conditions.

Aerenchyma developed during exposure to an hypoxic pre-treatment (Vartepatian et al. 2003) may increase the waterlogging tolerance of plants exposed to a subsequent waterlogging event. Root aerenchyma can not be produced when a plant is suddenly exposed to anoxic conditions, due to the complete lack of oxygen (Bragina et al. 2003, Evans 2003). Aerenchyma only develops when there is a gradual development of hypoxia.

The effect of flood pre-treatment on the response of three tree species to waterlogging was investigated by Anderson and Pezeshki (2001). It was reported that pre-treatment did not increase the root porosity during subsequent waterlogging, but may have accelerated the development of highly porous roots. A study of the relationship between waterlogging tolerance and aerenchyma development in *E. globulus* and *E. nitens* (Connell 1998) showed waterlogging tolerance of both species may be enhanced by the induction of aerenchymatous root development prior to exposure to a second waterlogging event.

Hypotheses tested:

1. Hypoxic pre-conditioning will increase the tolerance of *E. globulus* and *E. nitens* to waterlogging, manifested as higher growth rates and increased degree of morphological adaptation, including the development of aerenchymatous roots.
2. Aerenchymatous root development will increase with the length of exposure to hypoxic pre-conditioning.
3. Root aerenchyma induced during hypoxic pre-conditioning will be retained during freely-drained conditions and confer a growth advantage to seedlings during a second waterlogging event.
4. The ability for recovery from waterlogging, manifested as higher growth rates after exposure to waterlogging, is an important measure of tolerance in *E. globulus* and *E. nitens*.

5.2 Materials and methods

5.2.1 Experimental design

Seedling Stock

One hundred and twenty *E. globulus* and *E. nitens* seedlings approximately 10 cm in height were supplied by Forestry Tasmania's Perth nursery in April 2004.

Potting Conditions

Seedlings of each species were transplanted into trays of plastic forestry tubes (tube dimensions were 4 x 4 cm and 7 cm deep), with 15 tubes per tray. Each treatment was represented by one tray of seedlings. Seedlings were given an establishment period of 20 days growth under glasshouse conditions before being subjected to the following treatments.

Treatments

The following 7 treatments were applied to both *E. globulus* and *E. nitens*;

1. **Control (drained)** - no preconditioning / non-waterlogged
2. **Control (waterlogged)** - no preconditioning / waterlogged
3. **4 x 3 days** - four cycles of three days under hypoxic preconditioning alternated with four days of drained conditions prior to waterlogging
4. **7 days** - one cycle of seven days of hypoxic preconditioning prior to waterlogging
5. **2 x 7 days** - two cycles of seven days of hypoxic preconditioning alternated with two cycles of seven days of drained conditions prior to waterlogging
6. **14 days** - one cycle of 14 days of hypoxic preconditioning prior to waterlogging
7. **1 month** - 28 days of hypoxic preconditioning prior to waterlogging

During the experiment, the mean day and night summer temperature; 24°C and 17.5°C. Mean day and night winter temperatures; 17.4°C and 10.5°C.

Hypoxic preconditioning was applied by submerging the seedling trays in a 10 cm deep plastic-lined steel tray filled with tap water. The water level was maintained at the soil surface within the tubes. Water was changed weekly. Hypoxic preconditioning treatments were applied so that all treatments were removed from preconditioning on the same day (day 28).

On completion of the preconditioning treatment each seedling was planted into a 4.5 L pot filled with potting mix. Native soil mix (4:1, pinebark:coarse sand) was supplied by Horticultural Supplies (Hobart, Tasmania). The base of each pot was covered with a 50 mm layer of gravel to facilitate drainage. Pots were filled with native soil mix to within 50 mm of the top. Seedlings were staked and randomised within the glasshouse and allowed to grow for nine weeks prior to the application of a second period of waterlogging.

The second period of waterlogging was applied by submerging the pots in 10 L plastic buckets filled with water. The water level was maintained at the soil surface for a period of nine weeks.

5.2.2 Measurements

Growth

Height

The heights of three seedlings per treatment were measured weekly throughout the experiment.

Biomass

Plant biomass harvests of three seedlings per treatment were conducted; (1) immediately prior to preconditioning; (2) on the day the preconditioning treatment ended; (3) at the end of the subsequent nine week growth period; (4) at the end of the second period of waterlogging.

Plant height, stem diameter, leaf number, length of primary and secondary (aerenchymatous) roots and the fresh and dry weights of root, stem and leaf were recorded.

Morphology

During the second period of waterlogging, the degree of wilting of leaves was quantified by recording the number of nodes wilted. Foliar chlorosis and necrosis and changes to pigment composition were visually assessed.

Physiology

Physiological measurements were conducted on three seedlings from each treatment (i) at the completion of the nine week growth period that followed the pre-conditioning, (ii) after three weeks of the second period of waterlogging and (iii) at the completion of the second period of waterlogging (nine weeks).

Predawn leaf water potential was measured using a pressure chamber (PMS Instrument Co., Corvallis, Oregon) (Scholander et al. 1965). A mature leaf from the lowest third of the crown was used for each measurement.

Diurnal photosynthesis and stomatal conductance measurements were conducted on control drained and control waterlogged seedlings of both species from 8 am to 4 pm, using the portable photosynthesis system (Li-COR, Li-6400) on 25 August 2003, after three weeks of waterlogging.

Predawn chlorophyll fluorescence was measured on the most recently expanded leaves of seedlings using the portable PAM-2000 fluorometer (Heinz Walz GmbH, Efeltrich, Germany) and 2030-B leaf-clip.

5.2.3 Statistical analyses

A 2-way ANOVA using the Proc and GLM procedures of SAS (SAS Institute, 1996) was conducted to analyse the effects of species and hypoxic preconditioning treatment on biomass variables at harvest 2 and 3. At harvest 3, a 3-way ANOVA was used to identify effects of species, pre-conditioning and waterlogging on the biomass of harvested seedlings. T-tests were conducted on photosynthetic data to compare species and waterlogging treatments.

A one-sided Dunnett's test (SAS Institute, 1996) was used to compare the final heights of seedlings in hypoxic pretreatment and the waterlogged control.

5.3 Results

5.3.1 Growth

Height

There were no significant differences in seedling height between pre-conditioned treatments during the pre-conditioning, nine week growth period or the second waterlogging period for either species (Figure 5.1). However, the height of drained seedlings was significantly greater than waterlogged seedlings after three weeks of the second waterlogging period in both species.

At the end of the four week recovery period, drained seedlings had significantly higher final heights in both species. Significant differences in the mean seedling height between pre-conditioning treatments were also evident. In *E. nitens* all pre-conditioning treatments, except the 14 day treatment, resulted in an increased plant height at the completion of the recovery period, with the 2x7 day treatment significantly different from the waterlogged control ($P = 0.03$). In *E. globulus*, 1 month preconditioning significantly increased seedling height relative to non-preconditioned treatments ($P = 0.04$).

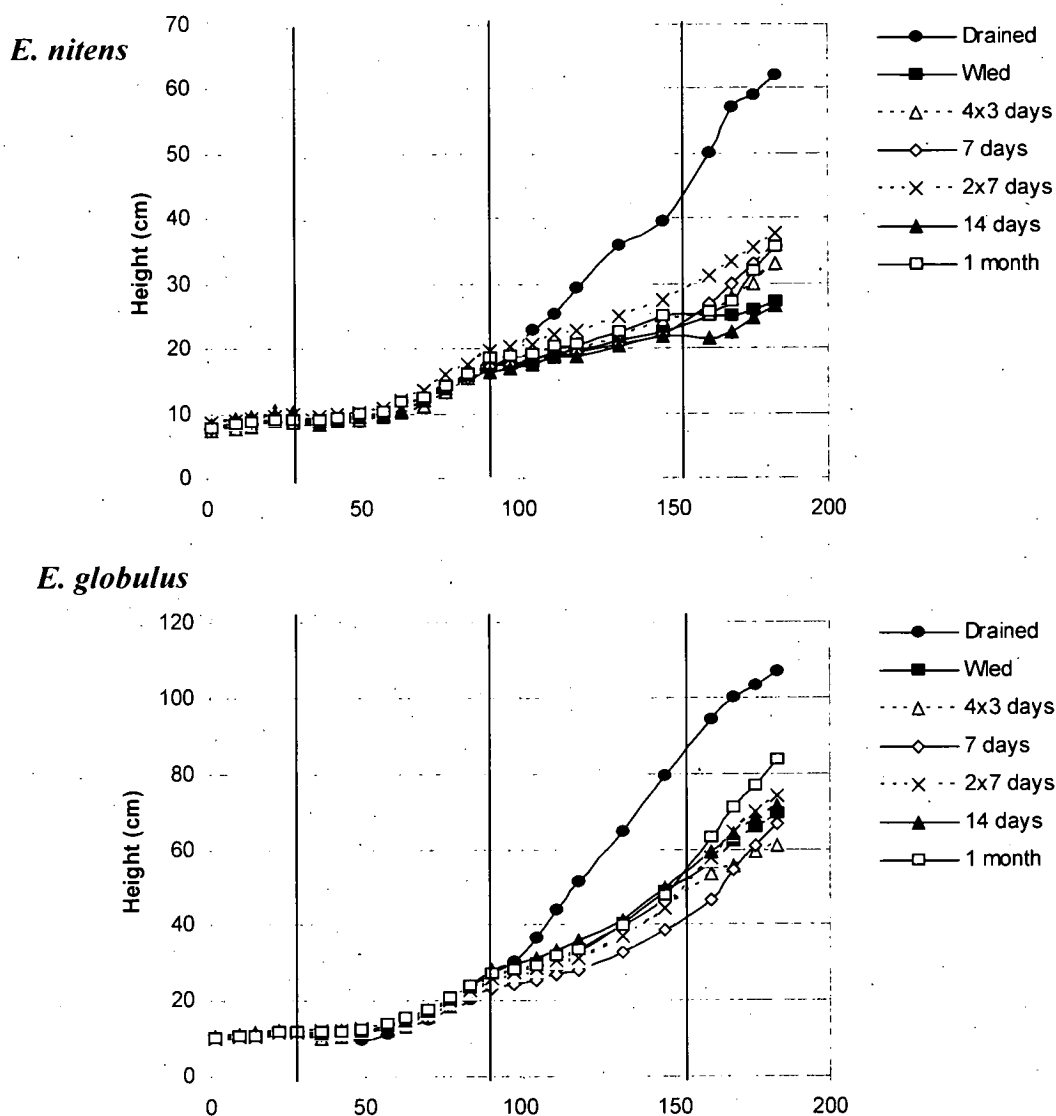


Figure 5.1 Mean seedling height of *E. nitens* (above) and *E. globulus* (below) over the experimental period. The end of the hypoxic preconditioning was at day 28; end of nine week growth period at day 91; and end of the nine week period of waterlogging at day 154.

Biomass

Prior to the application of pre-conditioning treatments, *E. globulus* had a significantly ($P < 0.05$) greater height, stem diameter and total dry weight than *E. nitens*, because the stock supplied was different in size.

At harvest 2 (after the 28 days of hypoxic preconditioning), the effect of species was still highly significant (Table 5.1). There was also a significant effect of pre-conditioning treatment on seedling height, stem diameter, root length, stem fresh weight, root fresh weight, fresh weight of aerenchymatous roots, leaf fresh and

dry weights and root:shoot ratio (Table 5.1). The only significant interaction between species and treatment was in leaf dry weight.

Table 5.1 Significance levels (P values < 0.05) of species and pre-conditioning treatment on the growth variables (height, stem diameter, leaf number, root length and dry and fresh weights of biomass components) of *E. globulus* and *E. nitens* at harvest 2 (after 28 days of hypoxic preconditioning).

Variable	Species	Treatment	Species x Treatment
Height	0.0002	0.0128	
Stem diameter	<0.0001	0.0525	
Leaf number	0.0144		
Root length		0.0332	
Stem fwgt	0.0087	0.0535	
Stem dwgt			
Root fwgt	0.0002	0.0385	
Root dwgt	0.0061		
Aerenchyma fwgt		0.0071	
Leaf fwgt	0.0014	0.0072	
Leaf dwgt	0.0003	0.0129	0.0531
Plant dwgt	0.0008		
Root:shoot ratio		0.0003	

Eucalyptus globulus only produced aerenchymatous roots under long periods of hypoxia, in the 2 x 7 day, 14 day and 1 month preconditioning treatments (Figure 5.2). In comparison, *E. nitens* developed aerenchymatous roots under all preconditioning conditions. For *E. nitens*, the highest percentages of aerenchymatous roots were found in the 4x3 and 28 day treatments (Figure 5.2).

Seedlings from the control treatment had the lowest root:shoot ratio of 0.34 in *E. globulus* and 0.32 in *E. nitens* (Figure 5.3). The root:shoot ratio generally increased with increased exposure to hypoxic preconditioning in *E. globulus*.

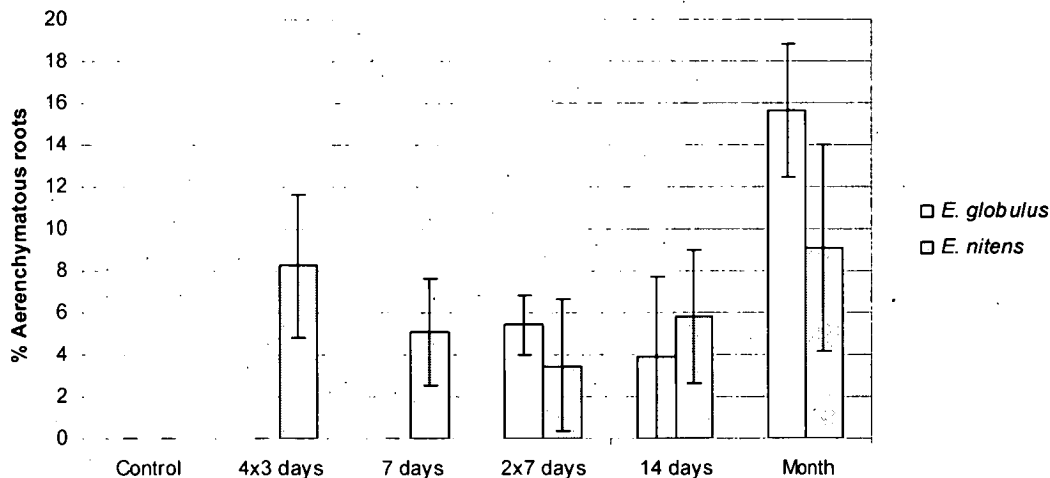


Figure 5.2 Mean percentage aerenchymatous root weight of *E. globulus* and *E. nitens* at harvest 2 (after 28 days of hypoxic preconditioning). Bars represent standard error.

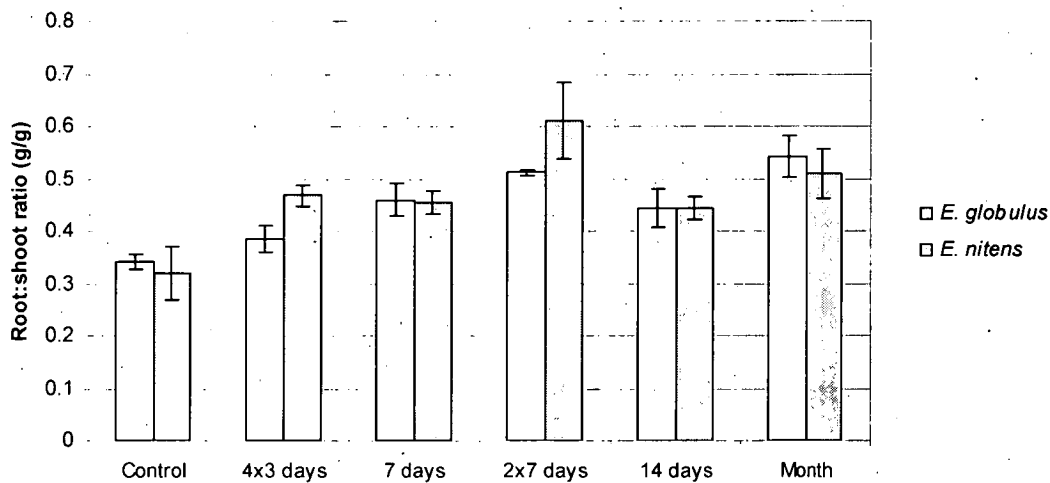


Figure 5.3 Mean total seedling root:shoot ratio (dry weight) of *E. globulus* and *E. nitens* at harvest 2 (after 28 days of hypoxic preconditioning). Bars represent standard error.

During the nine week growth period after preconditioning, the effect of treatment declined so the only significant effect was on seedling leaf number (Table 5.2). There was still a highly significant effect of species on plant height, leaf number, stem fresh and dry weights, fresh weight of aerenchymatous roots, leaf fresh and dry weights and total plant dry weight, due to differences in initial plant height.

Table 5.2 Significant effects (P values < 0.05) of species and preconditioning treatment on the growth variables (height, stem diameter, leaf number, root length and dry and fresh weights of biomass components) of *E. globulus* and *E. nitens* at harvest 3 (after nine week growth period).

Variable	Species	Treatment
Height	<0.0001	
Stem diameter		
Leaf number	0.0003	0.0417
Stem fwgt	0.001	
Stem dwgt	0.0001	
Root fwgt		
Root dwgt		
Aerenchyma fwgt	0.0032	
Percentage aerenchyma	0.0014	
Leaf fwgt	0.0004	
Leaf dwgt	0.0012	
Plant dwgt	0.0008	
Root:shoot ratio		

The percentage of aerenchymatous roots increased in all seedlings over the nine week growth period (up to day 91) (Figure 5.4). *Eucalyptus globulus* had developed aerenchyma in all treatments and had significantly greater aerenchymatous root development than *E. nitens*. The percentage of aerenchyma in *E. globulus* increased with increased length of hypoxic preconditioning treatment, similarly to the results from harvest 2 (day 28). There was no significant effect of treatment on the percentage of aerenchyma development in *E. nitens* (Figure 5.4).

During the freely drained growth period after hypoxic preconditioning, the root:shoot ratio of all treatments decreased (Figures 5.3 and 5.5). There were no significant differences between species.

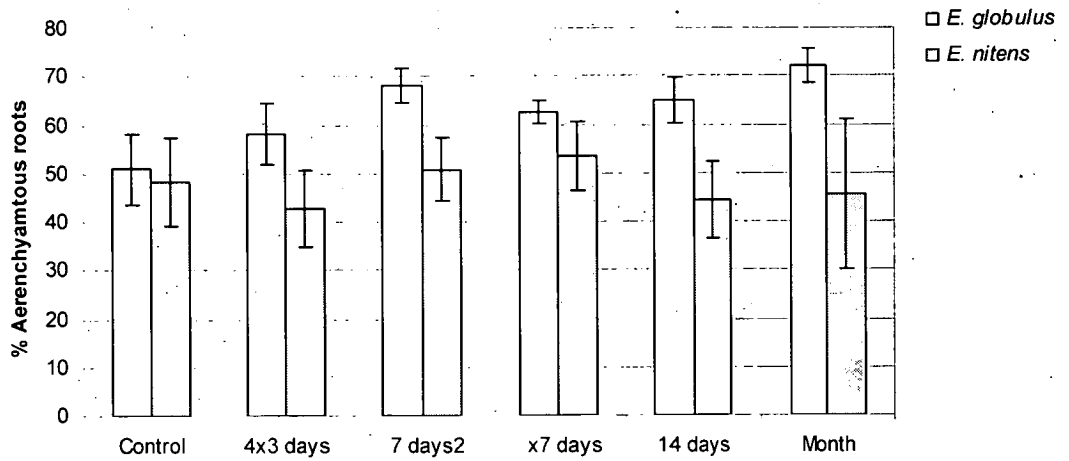


Figure 5.4 Mean percentage aerenchymatous root fresh weight of *E. globulus* and *E. nitens* at harvest 3 (after nine weeks growth period, day 91). Bars represent standard error.

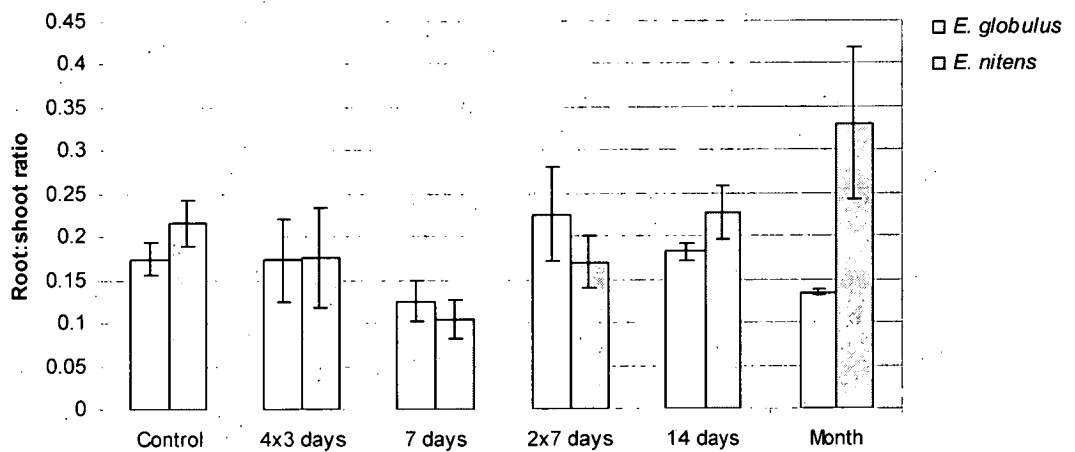


Figure 5.5 Mean total seedling root:shoot ratio (dry weight) of *E. globulus* and *E. nitens* at harvest 3 (after nine week growth period, day 91). Bars represent standard error.

Table 5.3 Significant effect (P values < 0.05) of species and hypoxic preconditioning treatment on the growth variables (height, stem diameter, leaf number, root length and dry and fresh weights of biomass components) of *E. globulus* and *E. nitens* at harvest 4 (after second waterlogging period, day 154).

Variable	Species	Water	Spp x Water	Treatment	Spp x Treatment
Height	<0.0001	<0.0001			
Stele diameter	<0.0001	0.0051			
Stem diameter	<0.0001	0.0002			
Leaf number	<0.0001	0.0046			
Stem fwgt	<0.0001	<0.0001			
Stem dwgt	<0.0001	<0.0001			
Leaf fwgt	<0.0001	<0.0001			
Leaf dwgt	<0.0001	<0.0001			
Root fwgt	0.0003	<0.0001	0.0003		0.0376
Root dwgt	0.0021	<0.0001	0.0206		
Aerenchyma fwgt	0.0002		0.0049		
% Aerenchyma	0.0079	<0.0001			
Plant dwgt	<0.0001	<0.0001			
Root:shoot ratio	0.0451	0.0045	0.0008		
Primary root length		<0.0001		0.0154	
Secondary root length	0.0092	0.0070	0.0291		

At the final harvest (after second period of waterlogging), the effect of species was highly significant across all growth variables (except primary root length) (Table 5.3). Waterlogging had a significant negative effect across all growth parameters, except in the development of aerenchymatous roots. The interaction between species and waterlogging was significant for root weight, fresh weight of aerenchymatous roots, root:shoot ratio and secondary root length.

The development of aerenchymatous roots was significantly higher in *E. globulus* than *E. nitens* (Figure 5.6). There was no effect of preconditioning treatments on the percentage of aerenchymatous roots for *E. globulus*. More than 80 % (a significant increase from harvest 3) of the fresh weight of the root system was aerenchymatous roots in all waterlogged plants (Plate 5.1 and 5.2).

Waterlogging also had a significantly positive effect on the development of aerenchymatous roots in *E. nitens*. There was an increase in the percentage of aerenchymatous roots of 4x3 day and 2x7 day pretreated seedlings compared to the non preconditioned waterlogged control (Figure 5.6).

The percentage of aerenchymatous roots increased during the nine week second waterlogging period in both species (Figures 5.4 and 5.6)

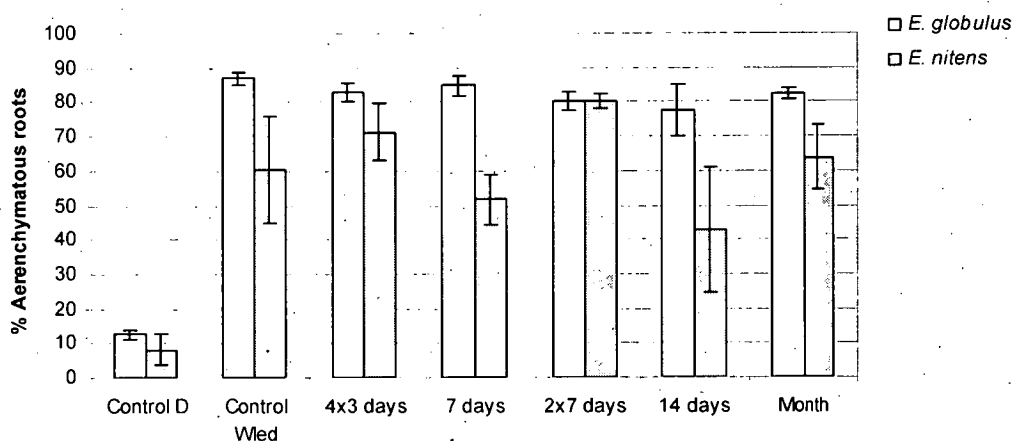


Figure 5.6 Mean percentage aerenchymatous root fresh weight of *E. globulus* and *E. nitens* at harvest 4 (after nine weeks waterlogging, day 154). Bars represent standard error.

A significant effect of species ($P = 0.045$) on the root:shoot ratio at harvest 4 (after nine weeks of waterlogging) was evident. Waterlogged *E. globulus* had a significantly higher root:shoot ratio than waterlogged *E. nitens* in most treatments, through the development of an extensive aerenchymatous root system (Figure 5.7). There was no significant effect of hypoxic preconditioning on the final root:shoot ratio of waterlogged *E. globulus* seedlings. The significant interaction between species and waterlogging ($P = 0.0008$) indicates that the two species

responded differently to the second waterlogging period. In *E. globulus*, waterlogging induced an increase in root:shoot ratio, whereas, in *E. nitens*, waterlogging significantly reduced the root:shoot ratio across all treatments (Figure 5.7).

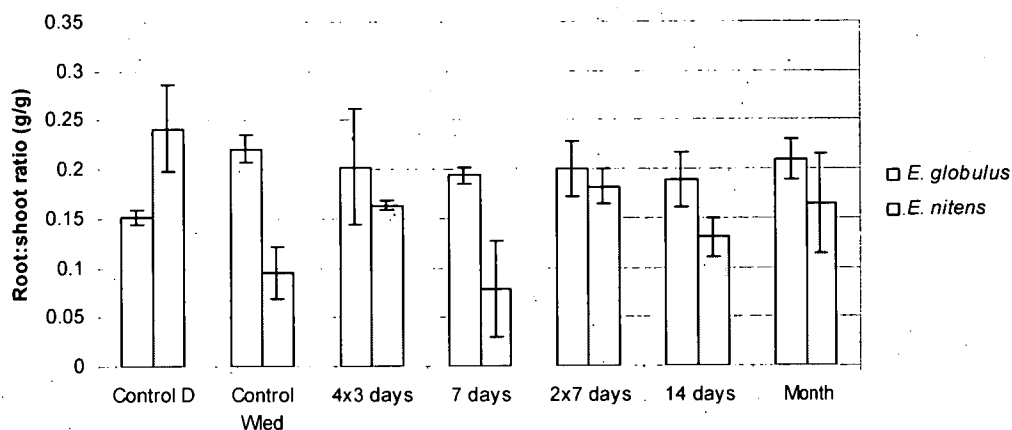


Figure 5.7 Mean total seedling root:shoot ratio (dry weight) of *E. globulus* and *E. nitens* at harvest 4 (after nine weeks waterlogging, day 154). Bars represent standard error.

Waterlogging *E. globulus* seedlings had significantly increased secondary root length compared to drained control (Figure 5.8). *Eucalyptus globulus* had a significantly longer secondary root length than *E. nitens* in all waterlogged treatments ($P = 0.0092$) (Figure 5.8). For *E. nitens* only the 1 month preconditioning treatment increased secondary root length over the drained control.

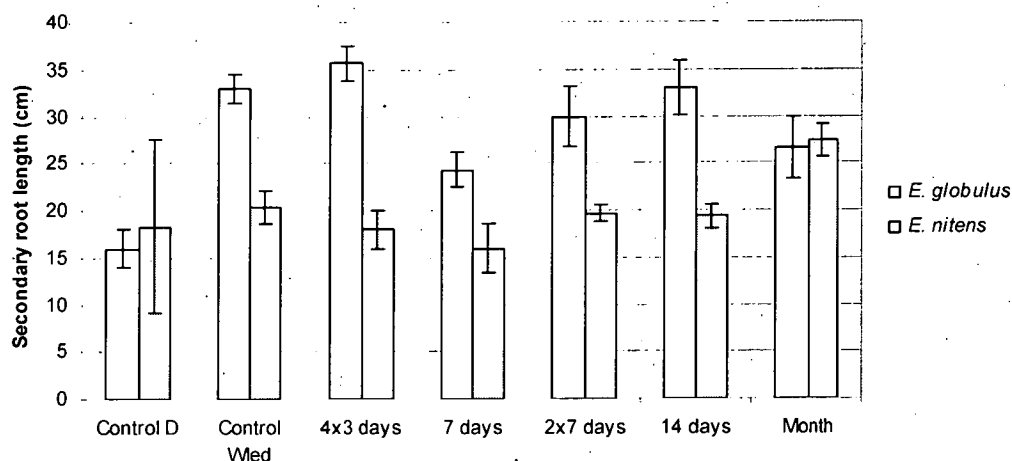


Figure 5.8 Mean total seedling secondary root length of *E. globulus* and *E. nitens* at harvest 4 (after nine weeks waterlogging, day 154). Bars represent standard error.



Plate 5.1 Typical example of aerenchymatous root development in *E. globulus* after nine weeks of waterlogging, preconditioned with 28 days of hypoxia.



Plate 5.2 Typical example of aerenchymatous root development in *E. nitens* after nine weeks of waterlogging, preconditioned with 28 days of hypoxia.

5.3.2 Physiology

Leaf water potential

There were no significant differences between the drained and waterlogged seedlings of either species throughout the experimental period (Figure 5.9).

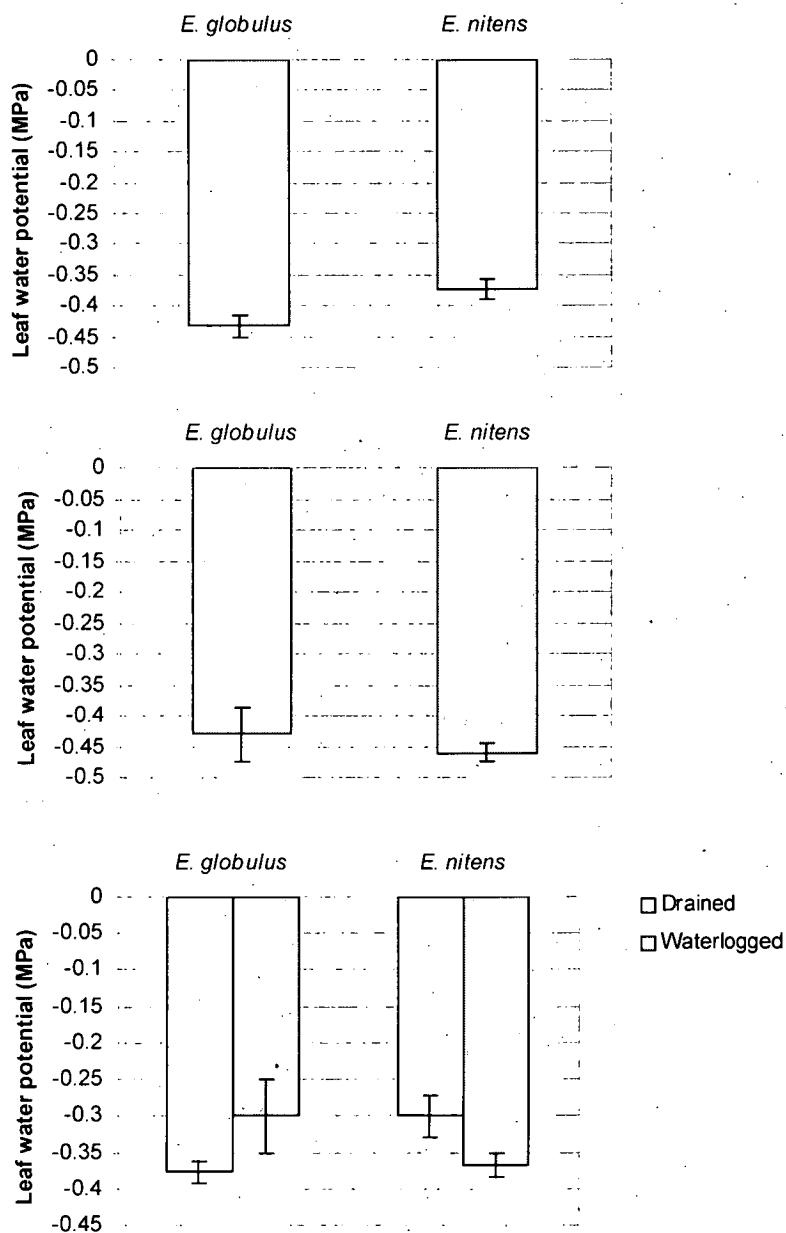


Figure 5.9 Predawn leaf water potential of *E. globulus* and *E. nitens* prior to waterlogging (above), after three weeks of waterlogging (centre) (waterlogged only) and after nine weeks of waterlogging (below). Bars represent standard error.

Photochemical efficiency

Prior to the application of waterlogging, there was no significant difference between the two species in their predawn F_v/F_m ratios (Figure 5.10).

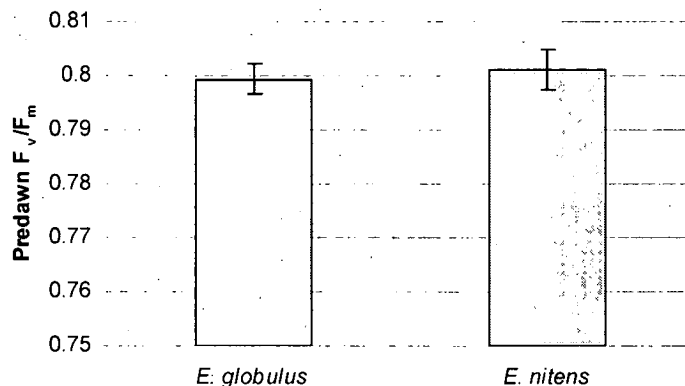


Figure 5.10 Predawn leaf F_v/F_m of *E. globulus* and *E. nitens* after nine week growth period (day 91). Bars represent standard error.

After nine weeks of waterlogging, the F_v/F_m ratio was above 0.77 in all treatments. There was a positive effect of waterlogging on the predawn F_v/F_m ratio of both species after nine weeks of waterlogging (Figure 5.11). *Eucalyptus globulus* seedlings had lower F_v/F_m ratios than *E. nitens* across all treatments.

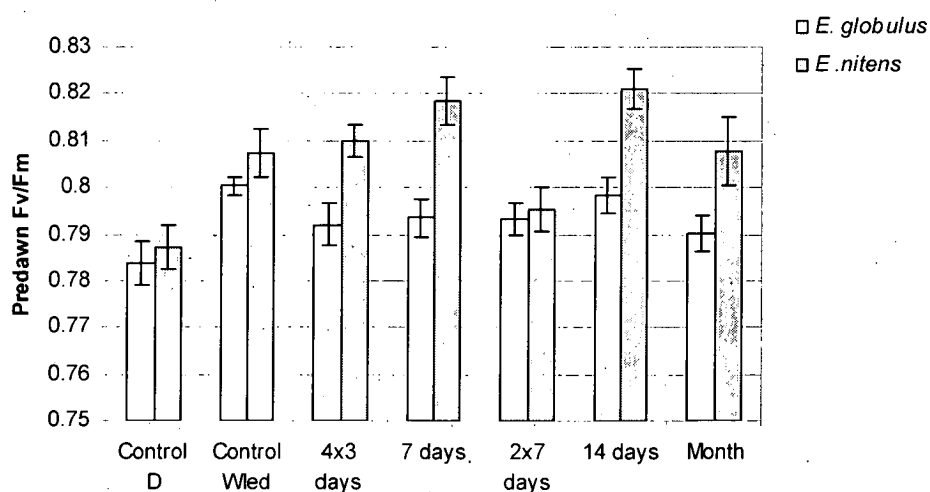


Figure 5.11 Predawn F_v/F_m of *E. globulus* and *E. nitens* after nine weeks of waterlogging (day 154). Bars represent standard error.

Photosynthesis and stomatal conductance

After three weeks of waterlogging, the net photosynthetic rate of *E. globulus* drained seedlings reached a higher midday maximum than *E. nitens* (Figure 5.12). The photosynthetic rate of waterlogged *E. globulus* seedlings showed no diurnal response, with a rate of approximately $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ maintained throughout the six hour measurement period. There was a significant difference in the morning net photosynthetic rate (at 8 am) of waterlogged *E. globulus* and *E. nitens* seedlings. The photosynthetic rate of waterlogged *E. nitens* seedlings declined from 8 am to 12 pm, before increasing after midday to an average of $7.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was maintained to 4 pm.

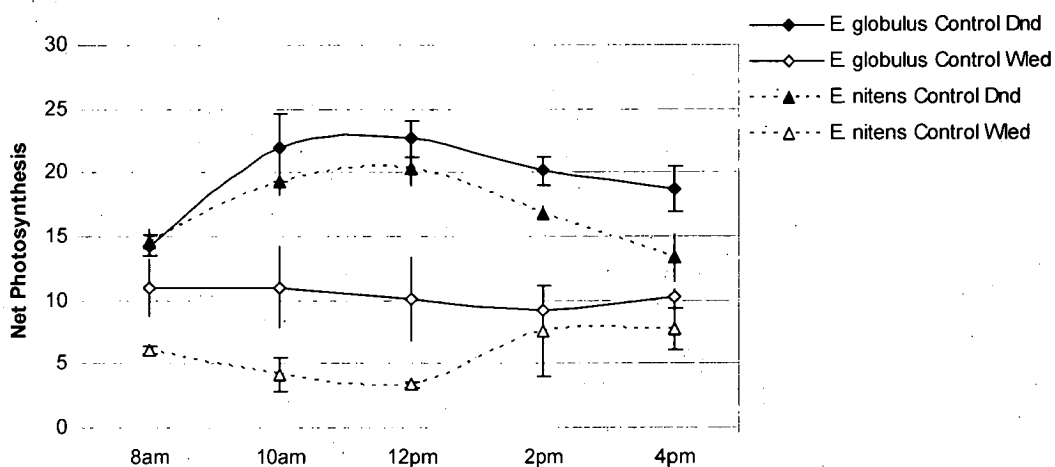


Figure 5.12 Diurnal photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of *E. globulus* (above) and *E. nitens* (below) drained and waterlogged seedlings after three weeks of waterlogging, day 112. Bars represent standard error.

The stomatal conductance (Figure 5.13) of seedlings showed a similar trend to net photosynthesis. Drained *E. globulus* seedlings had higher stomatal conductance than *E. nitens* throughout the day, with the peak of 0.52 and $0.48 \text{ mol m}^{-2} \text{s}^{-1}$ respectively, occurring at 10 am, after which the stomatal conductance steadily decreased. Waterlogging had a significantly negative effect on the stomatal conductance of both species, with waterlogged *E. globulus* seedlings maintaining a significantly higher stomatal conductance than *E. nitens* throughout the day, with a steady decrease from 8 am.

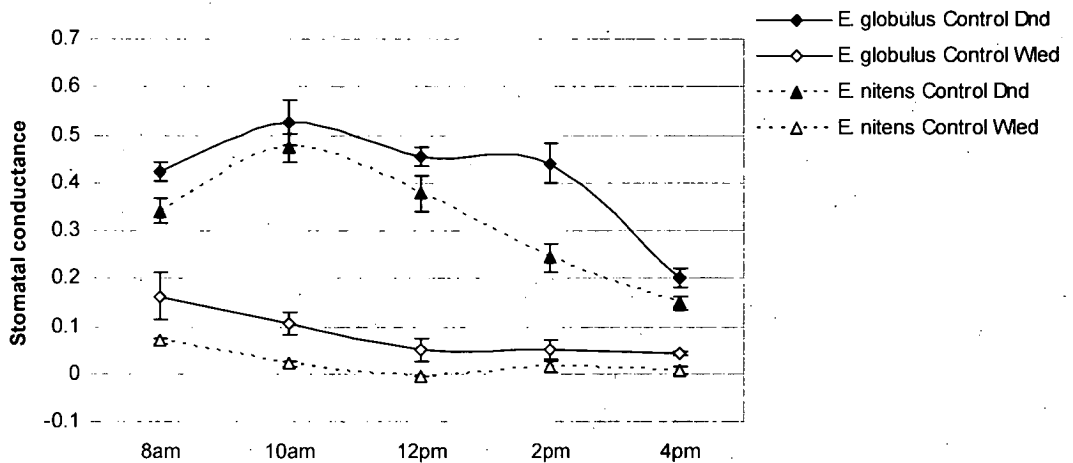


Figure 5.13 Diurnal stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) of *E. globulus* and *E. nitens* drained and waterlogged seedlings after three weeks of waterlogging, day 112. Bars represent standard error.

5.3.3 Morphology

Wilting

After 24 hours of the second period of waterlogging, wilting of both species was observed (Plates 5.3). Wilting was observed to begin after sunrise and end by sunset. Wilting was noted to occur up to the second and third nodes of most waterlogged seedlings, but up to the sixth node in some individuals.

Recovery of leaf turgor was noted to occur in *E. globulus* first, usually by 1 pm.

The waterlogged *E. nitens* seedlings did not recover until after 4 pm.

Foliar chlorosis

The development of inter-veinal chlorosis of waterlogged seedlings was observed (Plate 5.4).

Aerotropic root development

The development of aerenchymatous roots above the soil surface (aerial) was noted in *E. globulus* seedlings after nine weeks of waterlogging (Plate 5.5).



Plate 5.3 Waterlogged *E. globulus* (left) and *E. nitens* (right) seedlings showing typical wilted response after 5 days of waterlogging.



Plate 5.4 *Eucalyptus globulus* seedling after nine weeks of waterlogging with typical interveinal chlorosis.



Plate 5.5 Aerenchymatous root development on the surface of the soil in *E. globulus* after nine weeks of waterlogging.

5.4 Discussion

Tolerance to waterlogging is characterised by the maintenance of relatively high growth rates, morphological adaptation and the recovery of physiological and metabolic processes upon re-aeration (Gibbs and Greenway 2003). The ability to recover from transient waterlogging or periods of hypoxia of the root system was crucial in evaluating the waterlogging tolerance of plant species (Kriezeck 1982, Malik et al. 2001). The tolerance of eucalypt species to waterlogging has been demonstrated to be strongly related to the production of morphological and anatomical adaptations (Clemens et al. 1978, Connell 1998). The typical morphological response of many eucalypt species, including *E. camaldulensis*, *E. globulus* (Sena Gomes and Kozlowski 1980), *E. viminalis*, *E. ovata*, *E. robusta* (Ladiges and Kelso 1977, Clemens and Pearson 1977), *E. grandis* and *E. saligna* (Clemens et al. 1978) to waterlogging is characterised by the development of adventitious and aerenchymatous roots. The induction of adaptive root features during hypoxic preconditioning may increase the survival and growth of eucalypt seedlings to subsequent waterlogging episodes.

The use of hypoxic preconditioning to increase the survival and tolerance of waterlogged plants has been demonstrated in a number of crop species, including maize (Andrews et al. 1994, Bragina et al. 2003, Johnson et al. 1989) and rice (Ellis and Setter 1999). Waterlogging pretreatment has been demonstrated to increase the survival and growth of a number of eucalypt species (*E. camaldulensis*, *E. tereticornis*, *E. robusta* and *E. globulus*) under subsequent saline waterlogging (Marcar 1993).

The survival and growth of a number of Tasmanian tree species to waterlogging has been shown to be affected by the periodicity of waterlogging (Pryor et al. 2005, in press). The waterlogging-tolerant species, *Leptospermum scoparium* and *Acacia melanoxylon* were tolerant of a range of waterlogging periodicities, ranging from cycles of 15, 30 and 60 days. In comparison, *E. obliqua* seedlings were only tolerant (in terms of survival and growth) of longer periods of exposure to waterlogging (60 days). The degree of aerenchymatous root development was

associated with greater survival in these species (Pryor et al. 2005, in press). The ability of a species to morphologically adapt to waterlogged conditions and the retention of these features during non-waterlogged periods are the key factors responsible for the level of tolerance observed, and are affected by waterlogging intensity and duration.

It is important to monitor the growth of waterlogged seedlings throughout the waterlogging and re-aeration periods, in order to determine the performance and relative tolerance of the species to waterlogging (Malik et al. 2002) and the retention of adaptive features. Hypoxic preconditioning did not affect the survival or growth rate of either species during or immediately after the 28 day hypoxic pre-conditioning treatment. This result indicates that short term exposure to hypoxic conditions does not have a negative effect on seedling height, which may be an important factor in developing seedlings suitable for planting out in waterlogged areas. Significant effects of pre-conditioning on growth did start to become evident however, during the nine week growth period between preconditioning and the second waterlogging event, and more so during the four week recovery period. This result highlights the need to assess the growth rate of waterlogged seedlings during recovery from a waterlogging event, and this may be the most useful measure of determining a species' tolerance to waterlogging.

The critical length of hypoxic preconditioning required to increase waterlogging tolerance is highly variable between species, ranging from a few hours to days (Gibbs and Greenway 2003). The manipulation of the duration, frequency and timing of preconditioning treatments and use of seedlings of different ages may affect the success of preconditioning in increasing the tolerance of plant species to waterlogging (Andersen and Pezeshki 2001). The 2x7 day and 28 day preconditioning treatments were the most effective in *E. nitens* and *E. globulus* respectively, in terms of increasing the relative height and development of aerenchymatous roots under waterlogged conditions.

Aerenchyma production has been shown to increase plant tolerance and growth under waterlogged conditions (Akilan et al. 1997, Evans 2003, Poot and Lambers

2003) and has been demonstrated to be increased during acclimation to waterlogging in soybean (Bacanamwo and Purcell 1999). The application of hypoxic preconditioning was successful in inducing aerenchymatous root development in both species, with the level of aerenchyma production during the preconditioning and second waterlogging treatment closely related to seedling growth rate during these periods.

Exposure to hypoxic conditions induces the production of ethylene and the activation of hydrolytic enzymes, which are involved in cortical cell degradation and the formation of aerenchymatous tissue (Bragina et al. 2003). *Eucalyptus nitens* produced a relatively small amount of aerenchymatous root tissue during all preconditioning treatments. This result indicates the inherently higher capacity for aerenchymatous root development in *E. nitens* than *E. globulus*. The development of aerenchymatous roots in *E. globulus* was only evident after long-term hypoxic preconditioning (14 days or longer).

The continued growth and survival of the aerenchymatous root system during drained periods has been demonstrated in wheat (Malik et al. 2002). The retention of aerenchymatous root tissue in hypoxically pretreated seedlings during the freely drained period, prior to waterlogging, was an important result of this experiment. Control (non-preconditioned seedlings) of both species also developed an aerenchymatous root system during this period. This result is indicative of the inherent capacity of the two species to develop aerenchymatous roots, and that it may be dependent on the specific developmental age or size of the seedling (*E. nitens* ~ 18 cm and *E. globulus* ~ 25 cm). The percentage of aerenchymatous root significantly decreased over the nine week growth period, indicating that aerenchymatous root development may be an early response to establishment in these species. This is consistent with the results of Andersen and Pezeshki (2001) who reported that preconditioning did not increase the root porosity of three waterlogged tree species, but may have accelerated the process.

Waterlogging during vegetative growth is reported to be less detrimental to growth than waterlogging during reproductive phases (Umaharan et al. 1997).

Root length of waterlogged seedlings has been demonstrated to affect the development of highly porous aerenchymatous roots under hypoxic conditions (Thomson et al. 1990). Shorter roots have been reported to have a greater capacity for the development of aerenchyma. Therefore, exposing smaller/younger seedlings to hypoxic conditions may induce a greater level of aerenchymatous root development than would be possible at later developmental stages. Small eucalypt seedlings are generally planted out in the field from winter to early spring (Worledge et al. 1998), when there is a high risk of waterlogging. The significant loss in productivity of waterlogged trees has been demonstrated in Chapter 2.

This experiment has demonstrated that the induction of aerenchymatous roots in *E. globulus* and *E. nitens* is possible under specific hypoxic preconditioning treatment. Inducing the formation of a highly porous root system prior to planting seedlings into areas susceptible to waterlogging may significantly increase the tolerance of seedlings to waterlogging during the first year, and minimise the loss in productivity attributable to waterlogging in eucalypt plantations.

~ CHAPTER 6 ~

Response of *E. globulus* and *E. nitens* to hypoxia under hydroponic conditions and aerenchymatous root porosity**6.1 Introduction**

Waterlogged soils typically have very low concentrations of dissolved oxygen, due to a 10,000-fold reduction in the diffusion rate of oxygen in water compared to air. The formation of aerenchyma is regarded as an essential trait in maintaining root function under these conditions (Armstrong et al. 1983). The production of aerenchyma under hypoxic conditions is one of the most widely studied responses of plant species to waterlogging. Historically, the well-studied species are typical wetland species (Smirnoff and Crawford 1983, Justin and Armstrong 1987), or economically important crop species for example, wheat (Thomson et al. 1992), corn (Drew et al. 1979, Atwell et al. 1985), pea (Armstrong et al. 1982, 1983), and rice (Armstrong 1971, Colmer et al. 1998).

Aerenchyma is the development of extensive cortical air spaces in the root and stem, which provides a low resistance pathway for the internal diffusion of oxygen from the shoot to the root system (Gibberd et al. 1999). Root oxygen can then be utilised for respiration or be released from the root tissue, resulting in reoxidation of the rooting environment.

There are two mechanisms of aerenchyma development (Evans 2003);

1. Schizogenous – separation of cortical cells through differential development;
2. Lysigenous – cortical cell death, resulting in the development of large air spaces within the cortex

Root porosity is a measure of the relative volume of root tissue composed of air space. A strong correlation has been reported between root porosity and increased

root growth and survival under hypoxic conditions (Armstrong 1979, Gibberd et al. 2001, Thomson et al. 1990). The extent of development of root aerenchyma is used as a measure of a species' tolerance to waterlogging. Waterlogging-tolerant species have a greater capacity to develop aerenchyma than non-tolerant species, resulting in higher percentage root porosity.

Nutrient deficiency has been identified as a cause of waterlogging damage in a number of species including soybean (Bacanamwo and Purcell 1999), wheat (Malik et al. 2001), loblolly pine (Hook et al. 1983) and also in some eucalypt species (Van der Moezel et al. 1988, Close and Davidson 2003). Significant reductions in foliar nitrogen, phosphorus, magnesium, potassium and calcium have been reported for waterlogged *E. globulus* and *E. nitens*. Nutrient deficiency can develop through two processes, which may act in combination; 1) reduced availability of elements in their bioactive form under low dissolved oxygen concentrations and redox potentials in waterlogged conditions; 2) restricted uptake and transport of nutrients through the depletion of respiratory substrates within the root tissue, which occurs rapidly under anaerobic respiration compared to aerobic respiration, and root death. The primary mechanism through which waterlogged *E. globulus* and *E. nitens* seedlings develop foliar nutrient deficiency is unknown.

The extent of aerenchyma development in the root and stem, and the porosity of aerenchymatous roots are commonly used measures in interspecific comparisons of tolerance to waterlogging. Hydroponic culture systems have a number of advantages in these experiments. It is easier to assess aerenchyma development in roots grown in hypoxic nutrient culture rather than in soil systems, and patterns of root growth and anatomy studies are more easily conducted on the same individual during treatment. It is also easier to control nutrient and oxygen concentrations and the development of hypoxic conditions in an aqueous medium.

The ability of *E. globulus* and *E. nitens* to develop aerenchymatous roots with high root porosities under hypoxic conditions was examined to determine the relative tolerance of the two species to waterlogged conditions. By using well-

fertilised and non-fertilised seedlings the effect of nutrient status on aerenchyma development was assessed. The weekly renewal of nutrient solutions provided a continuous supply of essential elements to seedlings. By controlling the supply and availability of nutrients, the role of reduced root uptake in the development of low plant nutrient status was investigated.

Hypotheses tested;

1. *Eucalyptus globulus* has a greater capacity for aerenchyma development than *E. nitens* under hypoxic conditions.
2. *Eucalyptus globulus* and *E. nitens* seedlings grown under hypoxic conditions will develop foliar nutrient deficiencies.
3. Poor plant nutrient status will increase the development of root aerenchyma (and therefore increase root porosity) in seedlings grown in hypoxic conditions.
4. *Eucalyptus globulus* and *E. nitens* seedlings grown under hypoxic conditions will have a capacity to take up and accumulate nutrients from the rooting media, indicating that the primary cause of nutrient deficiency in waterlogged plants is reduced availability.

6.2 Materials and methods

6.2.1 Experimental design

Seedling stock

Thirty 20 cm tall *E. globulus* and *E. nitens* seedlings were sourced from Perth Forestry Tasmania nursery in January 2004 and were nutrient starved (non-fertilised) from January to December 2004. Thirty additional fertilised 15-20 cm tall *E. globulus* and *E. nitens* seedlings were supplied by the Forestry Tasmania Perth nursery in December 2004. All seedlings were re-potted and transferred to a glasshouse where they were grown from December 2004 to March 2005, under average day and night temperatures of 22.4°C and 16°C, respectively. During this period, high nutrient seedlings (supplied by the nursery in December 2004), were fertilised twice weekly with 100 mL of Peters Excel® solution, at a concentration of 1 g L⁻¹. The nutrient starved seedlings remained unfertilised.

At the end of the growth period (to March 2005) the mean heights of each treatment group were;

Non-fertilised *E. globulus* – 58 cm

Non-fertilised *E. nitens* – 34 cm

Fertilised *E. globulus* – 48 cm

Fertilised *E. nitens* – 66 cm

Plant culture

In February 2005, seedlings were transferred to solution culture. Two seedlings of the same species were placed in 3.5 L pots constructed from 150 mm PVC sewer pipe sealed with a base made from a sewer pipe end-cap. Each pot had a plastic push-on sewer end-cap lid with three 25 mm holes through which seedling root systems were passed. The stem was supported by non-absorbent cotton wool. The centre hole remained uncovered to facilitate gaseous exchange with the atmosphere.

The unfertilised seedlings were transferred to tap water and fertilised seedlings were grown in full-strength nutrient solution, the composition of which is shown in Table 6.1. The solution in all pots was renewed weekly throughout the

experiment. The pH of the solution was neutral (between 6.2 and 7.0) (Galloway and Davidson 1993).

Aeration of each pot was supplied by two 25 mm air stones, each on the end of a 30 cm length of rigid PVC tubing. The lines from the two air stones converged into one line with a small plastic tap, which allowed fine control of air flow. The line from each pot was connected to the main 10 mm (internal diameter) airline by a hydrodermic needle (size 25G, 0.5 x 25 mm). An Ingersoll-rand compressor maintained the air pressure in the mainline at 50 KPa resulting in an aeration rate of 500 mL min⁻¹ per pot. Seedlings were given an establishment period in aerated hydroponic culture of three months (February 2005 – May 2005) prior to the application of stagnant conditions.

Table 6.1 Composition of the nutrient solution, based on that described by Galloway and Davidson (1993).

Chemical	Final concentration	Stock (g/L)	Stock concentration	Final dilution (mL/L)
Sodium nitrate	4 mM	67.99	0.8 M	5
Potassium nitrate	3 mM	60.66	0.6 M	5
Magnesium sulphate	2 mM	104.8	0.4 M	5
Potassium chloride	1 mM	14.91	0.2 M	5
Ammonium nitrate	2 mM	32.02	0.4 M	5
Calcium nitrate	3 mM	141.7	0.6 M	5
Potassium dihydro-phosphate	0.2 mM	5.44	0.04 M	5
Boric acid	4.6 µM	0.0568	0.92 mM	5
Manganese chloride	0.5 µM	0.0198	0.1 mM	5
Copper sulphate	0.2 µM	0.01	0.04 mM	5
Zinc sulphate	0.2 µM	0.0116	0.04 mM	5
Ammonium molybdate	0.1 µM	0.0248	0.02 mM	5
Iron chelate	2 ppm	6.6	200 ppm	5

Treatments

The death of all fertilised *E. nitens* seedlings occurred rapidly after transfer to the hydroponic culture system. These seedlings had very low root:shoot ratios compared to other treatments. Due to the large leaf area of these seedlings, they quickly died of drought upon transfer to hydroponic culture.

Six replicates of fertilised and non-fertilised *E. globulus* and six replicates of non-fertilised *E. nitens* seedlings were selected from the surviving seedlings for experimentation, resulting in a total of 18 pots. The healthiest seedling from each pot was selected for experimentation. The second seedling was removed. All seedlings, including those that had been starved of nutrient, were then cultured in 3.5 L of full-strength nutrient solution (Table 6.1) to determine if seedlings growing in hypoxic conditions could take up nutrients from the rooting media.

Of the 18 pots, nine were randomly selected to become stagnant. The stagnant treatment was applied by de-oxygenating the solution by flushing with industrial dry nitrogen for 30 minutes, at a rate of 500 mL min⁻¹. Pots were then sealed and left to stagnate for a period of three weeks. All stagnant pots had agar added to solution at a concentration of 0.1% w/v to minimise solution convection. Stagnant pots were flushed twice weekly with nitrogen gas to maintain the oxygen concentration below 0.06 mol m⁻³. The remaining nine pots remained well aerated, with an oxygen concentration of > 0.3 mol m⁻³.

The dissolved oxygen concentration of the hydroponic solution was measured on days 1, 2, 3, 7, 10, 14 and 21 using a handheld dissolved oxygen – pH – mV – temperature meter; model WP-91 (TPS Pty Ltd.) (Table 6.2).

Table 6.2 Dissolved oxygen concentration (mol m^{-3}) during the three week stagnation period in aerated and stagnant solutions.

Day	Dissolved Oxygen Aerated (mol m^{-3})	Dissolved Oxygen Stagnated (mol m^{-3})
1	0.34	0.018
2	0.31	0.045
3	0.32	0.046
7	0.33	0.028
10	0.32	0.031
14	0.31	0.028
21	0.32	0.026

6.2.2 Measurements

Seedling height

The height of each seedling was recorded twice weekly throughout the three week stagnation treatment, and at each biomass harvest.

Biomass

Whole seedling harvests were conducted (i) immediately prior to transfer into hydroponic culture (March 2005); (ii) immediately prior to the application of stagnant treatment (May 2005) and (iii) at the completion of the three week stagnant treatment (June 2005). At each harvest, plant height, stem diameter, length of the longest root, leaf number and dry weight of root, stem and leaf were recorded.

Nutrition

Foliar nutrition was analysed from material collected at harvests 2 (after three months growth in aerated solution) and harvest 3 (after three weeks stagnation). Leaf material of three seedlings from each treatment was dried at 70°C (to constant weight), ground and analysed for the concentration of nitrogen,

phosphorus, potassium, magnesium and calcium using the sulphuric acid / hydrogen peroxide digest method (Lowther 1980).

Root porosity

The porosity of aerenchymatous roots from each seedling was calculated at the final harvest after three weeks of stagnation. The method used was modified from that described by Raskin (1983), using the equations given by Thomson et al. (1990). A 40 ml flask was filled with tap water at room temperature and closed with a rubber stopper with a metal hook attached. The flask was suspended from a balance and submerged in a water-filled 2 L flask, and the balance tared.

Root samples were carefully blotted with tissue to remove exterior water adhesion and cut into segments of approximately 50 mm. The fresh weight of each segment was weighed on an electronic balance (FW). Each segment was then individually placed in the submerged 40 mL flask and weighed (W1). Care was taken not to include trapped air bubbles. Roots were then infiltrated with tap water under near vacuum for a period of 10-15 minutes. The root sample was weighed for a second time (W2). The porosity of the root tissue was calculated as;

$$\text{Porosity} = 100 \cdot \frac{W2 - W1}{FW - W1} \quad (\% ; v:v)$$

6.2.3 Statistical analyses

A 3-way ANOVA using the proc and glm procedure of SAS (SAS Institute 1996) was conducted to analyse the effect of species, fertilisation and aeration on biomass harvest and root porosity data.

A 2-way ANOVA using the proc and glm procedures of SAS (SAS Institute 1996) was conducted to analyse the effect of nutrient status and aeration treatment on the final foliar nutrient concentrations of *E. globulus*. A 1-tailed T-test was conducted to test for a significant increase in the foliar nutrient concentration of non-fertilised stagnated *E. globulus* and *E. nitens* seedlings.

6.3 Results

6.3.1 Seedling growth

Height

There was no significant effect of stagnation on the growth rate of seedlings during the stagnant treatment (Table 6.3).

Table 6.3 Relative growth rate in height ($\text{cm cm}^{-1} \text{ week}^{-1}$) of *E. globulus* and *E. nitens* treatments during the three week stagnant treatment in hydroponic nutrient culture.

Mean standard error = $0.046 \text{ cm cm}^{-1} \text{ week}^{-1}$.

Treatment	<i>E. globulus</i>	<i>E. nitens</i>
Fertilised Aerated	2.86	N/A
Fertilised Stagnant	2.81	N/A
Non-fertilised Aerated	2.74	2.42
Non-fertilised Stagnant	2.74	2.45

Biomass

Fertilised seedlings were significantly larger than non-fertilised seedlings in all measured variables throughout the experiment. There was no significant effect of stagnation on seedling morphology.

6.3.2 Aerenchymatous root development and root porosity

E. globulus

Growth in stagnant solution increased the root porosity in *E. globulus* seedlings in both fertilised and non-fertilised seedlings (Table 6.4). At the end of the stagnation treatment, fertilised and non-fertilised *E. globulus* seedlings had similar root porosities (56 and 61 % respectively).

Fertilisation significantly decreased the aerenchymatous root porosity of *E. globulus* in aerated and stagnant treatments ($P = 0.0231$). Despite the higher root porosity of non-fertilised *E. globulus* seedlings, there was a greater development (dry weight) of aerenchymatous roots in fertilised treatments. Therefore, fertilisation results in a greater production of aerenchymatous root tissue, but with

lower porosity than the aerenchymatous roots in non-fertilised seedlings.

Eucalyptus globulus produced significantly more aerenchymatous root tissue than *E. nitens*.

E. nitens

Eucalyptus nitens had significantly greater mean root porosity than *E. globulus* ($P=0.0321$). Stagnation of *E. nitens* had the opposite effect than observed in *E. globulus* with a significant interaction between species and aeration ($P = 0.0357$). In the aerated treatment, *E. nitens* had more than twice the root porosity of *E. globulus*. In the stagnant treatment, the two species had identical root porosity values (61 %).

Table 6.4 Calculated root porosity and dry weight of aerenchymatous roots of *E. globulus* and *E. nitens* aerenchymatous roots from aerated, stagnant, non-fertilised and fertilised conditions at the end of the three week stagnation treatment.

Treatment	Mean root porosity (%) A	Mean Aerenchymatous root dry weight B
<i>E. globulus</i>		
Aerated Non-fertilised	39.799 \pm 17.573	1.64 \pm 0.41
Aerated Fertilised	19.710 \pm 4.112	3.04 \pm 1.01
Stagnant Non-fertilised	61.061 \pm 1.061	0.77 \pm 0.11
Stagnant Fertilised	56.504 \pm 6.175	1.66 \pm 0.41
<i>E. nitens</i>		
Aerated Non-fertilised	85.998 \pm 5.178	0.173 \pm 0.05
Stagnant Non-fertilised	61.489 \pm 5.276	0.699 \pm 0.14

6.3.3 Leaf Nutrition

E. globulus

Immediately prior to the imposition of the stagnation treatment, fertilised plants had significantly higher foliar nitrogen, phosphorus and potassium concentrations ($P < 0.01$), and decreased magnesium and calcium concentrations than unfertilised treatments (Figure 6.1) in *E. globulus*.

There were significant changes in foliar nutrition following the application of three weeks of full strength nutrient solution to all treatments (including unfertilised) and imposition of stagnant conditions.

Fertilised *E. globulus* seedlings had significantly higher nitrogen ($P = 0.001$) and potassium ($P = 0.0012$) concentrations than non-fertilised seedlings after the three week stagnation treatment. As expected, transferring non-fertilised seedlings to full strength solution resulted in an increase in foliar concentration of nitrogen, phosphorus and potassium, under aerated conditions in both species.

The transfer of non-fertilised seedlings into fertilised conditions increased foliar nutrition (except for magnesium) during the stagnation period (Figure 6.1).

Therefore, nutrient starved seedlings were capable of taking up and accumulating nutrients under stagnant conditions, but still had lower nutrient status than fertilised seedlings after three weeks of stagnation (Figures 6.1 and 6.2).

The effect of stagnation on fertilised *E. globulus* seedlings was to significantly reduce foliar nitrogen ($P = 0.0064$), phosphorus ($P = 0.033$) and potassium ($P = 0.0115$) concentrations relative to aerated treatments.

E. nitens

The concentration of foliar nitrogen, phosphorus, potassium, magnesium and calcium was analysed in non-fertilised seedlings only in *E. nitens* (Figure 6.2).

The results are consistent with those of *E. globulus*. *Eucalyptus nitens* had lower concentrations of all elements than *E. globulus* across all treatments and at both harvests.

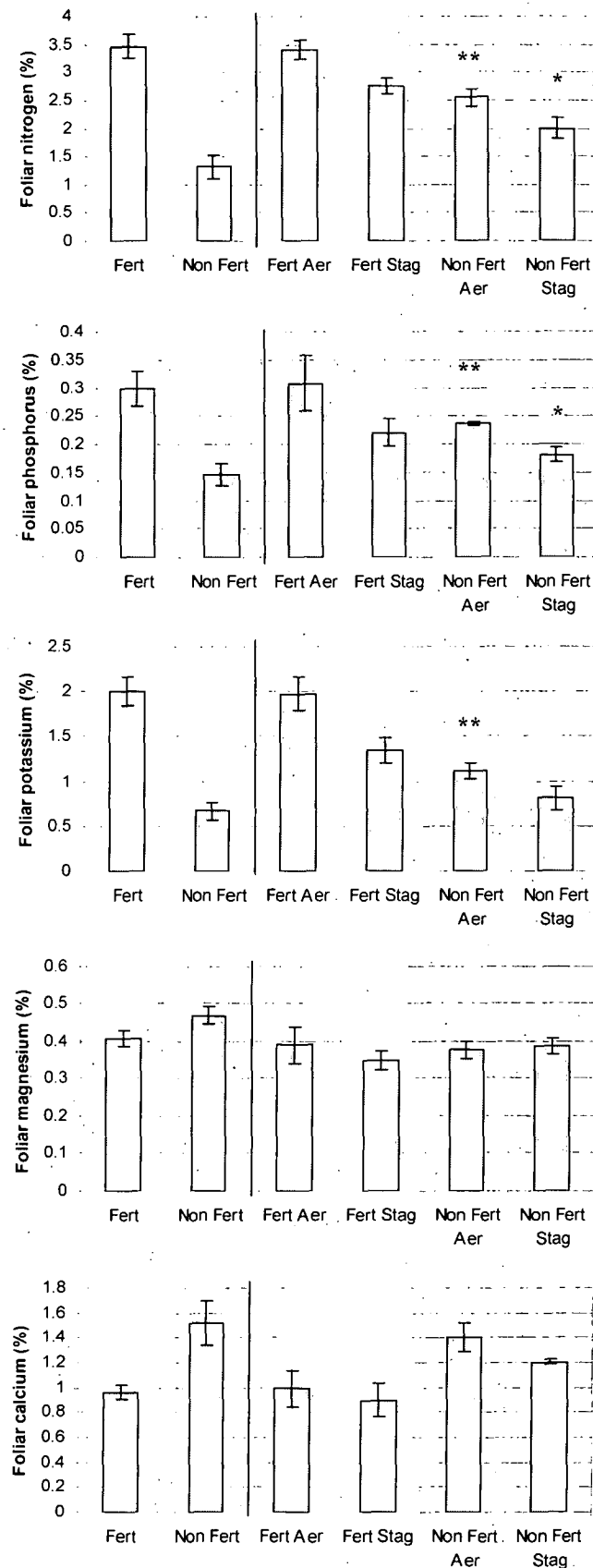


Figure 6.1 Mean foliar concentrations of nitrogen, phosphorus, potassium, magnesium and calcium in *E. globulus* seedlings prior to application of stagnant conditions (before line) and after 3 weeks of stagnant treatment. Fert = fertilised, Non Fert = non-fertilised, Aer = aerated solution and Stag = stagnated solution. Note that all treatments were cultured in full strength nutrient solution during the 3 week stagnation treatment. Significance levels * = $P < 0.1$, ** = $P < 0.01$ refer to the 1-tailed t-test for a significant increase in foliar concentration in non-fertilised seedlings. Bars represent standard error.

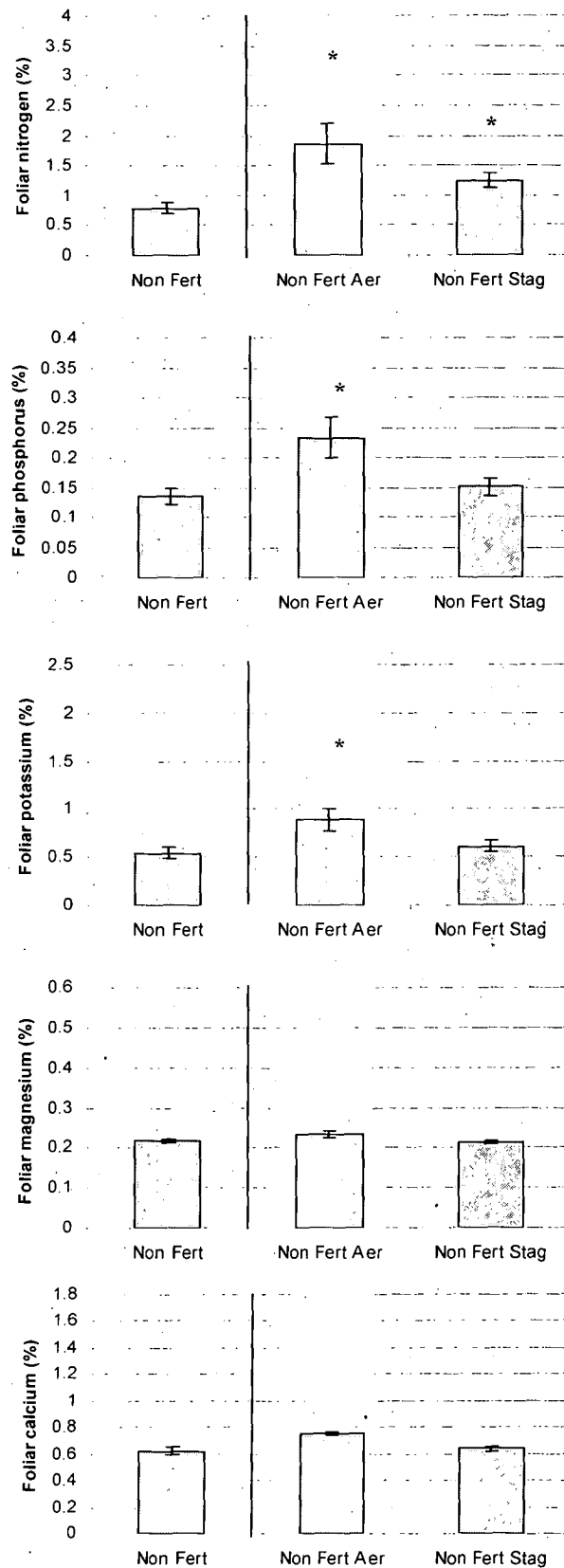


Figure 6.2 Mean foliar concentrations of nitrogen, phosphorus, potassium, magnesium and calcium in *E. nitens* seedlings prior to application of stagnant conditions (before line) and after 3 weeks of stagnant treatment. Fert = fertilised, Non Fert = non-fertilised, Aer = aerated solution and Stag = stagnated solution. Note that all treatments were cultured in full strength nutrient solution during the 3 week stagnation treatment. Significance levels * = $P < 0.1$, ** = $P < 0.01$ refer to the 1-tailed t-test for a significant increase in foliar concentration in non-fertilised seedlings. Bars represent standard error.

6.4 Discussion

Eucalyptus globulus had higher relative growth rates than *E. nitens* during hydroponic culture, indicating that *E. globulus* is inherently more tolerant of solution culture, which supports that described by Connell (1998). Fertilised *E. nitens* seedlings were incapable of surviving solution culture, due to an extremely low root:shoot ratio. Therefore, the comparison of seedlings with high and low nutrient status was only possible in *E. globulus*.

The capacity for aerenchymatous root development under waterlogged conditions has been reported to be greater in *E. globulus* than *E. nitens* (Connell 1998). In this experiment, *E. globulus* had a higher degree of aerenchyma development than *E. nitens*. Associated with the greater mass of aerenchymatous roots in fertilised *E. globulus* seedlings, the porosity of aerenchymatous roots was significantly less than that in non-fertilised seedlings. This result is important as it indicates the well-developed capacity for highly porous aerenchymatous root development in both *E. globulus* and *E. nitens*, and that higher root porosity develops in relatively small aerenchymatous root systems of these species. Fertiliser had the effect of increasing the physical development of aerenchymatous roots, but not root porosity. Therefore, using either of these measures of aerenchymatous root development in isolation may not provide an accurate means for assessing the relative tolerance of a species.

Aerenchymatous roots are an adaptive mechanism of waterlogged plants, with the degree of aerenchymatous root development indicative of a species' relative tolerance to waterlogging. In drained conditions, root aerenchyma is reported to develop under nutrient poor conditions (Bouranis et al. 2003, Fan et al. 2003, Huang et al. 1994). Aerenchymatous roots have been demonstrated to be equally as efficient in nutrient uptake as non-aerenchymatous roots (Lizaso et al. 2001), and may also reduce the demand for nutrients under waterlogged conditions (Fan et al. 2003). In this experiment, increased aerenchymatous root development was associated with high foliar nutrition. In previous chapters, aerenchymatous root

development has been shown to increase under waterlogged conditions with the application of fertiliser (chapters 4 and 5).

Stomatal closure is a common and early response to waterlogged conditions (Chen et al. 2002) and results in the reduction in transpiration rate. Xylem loading is significantly reduced due to the low rate of transpiration, and this process results in a reduction in the transport of nutrients and the development of a reduced foliar nutrient status (Malik et al. 2001). In conjunction with reduced transport of nutrients, the reduction in root uptake of essential nutrients is a common response of waterlogged plants (Bange et al. 2004, Drew and Sisworo 1979, Kozłowski 1997, Kreuzweiser et al. 2002), which may result in the development of foliar nutrient deficiencies in waterlogged plants.

In young plants, nutrient content is representative of the uptake rate (Hill et al. 2001). Therefore, the foliar analysis of seedlings conducted at the end of the hypoxic treatment is effectively a measure of the uptake rate of hypoxic seedlings. The development of reduced foliar nutrient status was indicated by evidence of premature leaf senescence and nutrient translocation, with leaf loss and chlorosis development in the lower leaves, which is a common response to nutrient deficiency in many plants (Grove et al. 1996). Hypoxia resulted in reduced foliar nutrient concentrations of nitrogen, phosphorus and potassium in fertilised *E. globulus* seedlings. This result indicates the rapid effect of hypoxic conditions on the nutrient status of eucalypt seedlings.

There was a significant effect of fertilisation on the foliar nitrogen, phosphorus and potassium concentrations in seedlings prior to hypoxia, resulted in increased growth of fertilised *E. globulus*.

The capacity of a species to maintain nutrient uptake under hypoxic conditions is a measure of tolerance, with in-tolerant species displaying significantly reduced nutrient uptake rates (Kozłowski 1997). Fertilisation applied during hypoxia (provided as full-strength nutrient solution) had a positive effect on seedling nutrition, with aerated and stagnant seedlings exhibiting significantly increased

foliar nutrient status at the end of the three week hypoxic treatment. This result indicates that *E. globulus* and *E. nitens* seedlings were capable of nutrient uptake, at a reduced rate, during exposure to hypoxic conditions, and supports the hypothesis that reduced nutrient availability is the principal cause of foliar deficiency in these species under waterlogged conditions.

~ CHAPTER 7 ~

General Discussion

This study aimed to compare the relative tolerance of the two major temperate eucalypt plantation species, *E. globulus* and *E. nitens*, to waterlogging. The growth response and physiological and morphological tolerance mechanisms of the two species were investigated to determine which species was more waterlogging-tolerant, and therefore more productive when planted into sites at a high risk of waterlogging. The relative role of reduced plant water status, photoinhibition and the development of reduced plant nutrient status, as mechanisms involved in the loss of productivity under waterlogged conditions were investigated.

The extensive expansion of the hardwood plantation estate across Tasmania in recent years has resulted in waterlogging becoming an increasingly common and significant risk to plantation productivity. The identification of sites susceptible to waterlogging is an important component of site suitability assessment. Currently, soil inspections are conducted at potential plantation sites. This study found that this technique is an effective means of identifying potential waterlogging problems, as losses in productivity of waterlogged trees were more closely related to the presence of soil greying and history of waterlogging than any other measured trait, including extent of current waterlogging and soil water chemistry (see Chapter 2).

At both field sites monitored during this study, waterlogging resulted in a 75 % loss in tree productivity over the initial three years of establishment. After this time, the relative growth rate of waterlogged trees increased to that of drained trees. Despite this increase in the relative growth rate of waterlogged trees after three years of growth, the difference in height and conic volume between drained and waterlogged trees continued to increase. Therefore, only short-term management of waterlogged trees during the first two to three years of plantation establishment may be required to ameliorate this loss. The susceptibility of young seedlings to waterlogging in the field led to the design of a number of glasshouse

experiments investigating the physiological and morphological response of *E. globulus* and *E. nitens* to waterlogging.

The high survival rate of waterlogged *E. globulus* and *E. nitens* seedlings exposed to long-term waterlogging, under glasshouse conditions, indicated both species had an inherently high capacity to survive long-term waterlogging. The regulation of growth rate is a universal indicator of the tolerance to a range of environmental stresses (Grove et al. 1996). The differences in the growth responses of *E. globulus* and *E. nitens* seedlings to waterlogging is a good indicator of the differences in tolerance of the two species. *Eucalyptus globulus* maintained a relatively high growth rate, particularly early in the waterlogging period whilst nutrient status was high. In comparison, *E. nitens* had a decreased growth rate immediately from the onset of waterlogging under fertilised conditions (Chapter 3).

Decreased photosynthetic rate is a typical early response to waterlogged conditions (Kozlowski 1984). *Eucalyptus globulus* had a more severe and rapid reduction in photosynthetic rate under waterlogged conditions than *E. nitens*. The photosynthetic rate of waterlogged *E. globulus* seedlings was significantly reduced after only three weeks of waterlogging in experiment 1 (see Chapter 3), while *E. nitens* showed no significant reduction in midday photosynthetic rate until after 18 weeks of waterlogging.

The decrease in photosynthetic capacity may be due to the degradation of chlorophyll and stomatal enzymes (Hortensteiner and Feller 2002). A rapid decrease in chlorophyll content of waterlogged plants may be indicative of the development of photoinhibition. Indeed, photoinhibition has been reported in response to waterlogging in *E. globulus* and *E. nitens* (Close and Davidson 2003) where waterlogged conditions induced nutrient deficiencies (principally in phosphorus) which caused the development of photoinhibition. However, in the current study there was no evidence of photoinhibition. Predawn F_v/F_m of waterlogged plants remained above 0.7 in both the field and glasshouse physiological studies under waterlogged conditions. Therefore, photoinhibition of

waterlogged plants was eliminated as the primary cause of waterlogging damage and reduction in growth.

In studies of plants under waterlogged conditions, which result in low root zone oxygen concentrations, there is a universal stomatal response (i.e. closure) but no consistent reduction in plant water potential (Sojka and Stolzy 1980, Nicolas et al. 2005). This study on *E. globulus* and *E. nitens* supported this conclusion. There was no indication that stomatal closure was associated with the development of water stress during waterlogging. Neither species exhibited significantly reduced predawn leaf water potential during long term waterlogging in the field, or under glasshouse conditions relative to the drained controls. This is consistent with results from a number of other studies on eucalypt species (Pereira and Kozlowski 1977, Close and Davidson 2003). Therefore, an alternative mechanism is responsible for the reduction in growth and physiological performance of these species.

Waterlogging has a dual effect on soil–plant nutrient relations. Saturation of the soil induces a series of chemical changes resulting in changes to the availability of many essential plant nutrients, including nitrogen, phosphorus, sulphur, zinc and manganese (Cresser et al. 1993, Ghanem et al. 1998, Phillips and Greenway 1998) (See Chapter 1). Fluctuations in water table height, which may be a seasonal event, result in the loss of available nitrogen, through sequential ammonification, nitrification and de-nitrification (Phillips 2001). Under waterlogged conditions, the primary source of nitrogen is ammonium (Belder et al. 2005). These changes will affect the ability of *E. globulus* and *E. nitens* to tolerate waterlogging, as both these species have a preference for ammonium over nitrate as the primary nitrogen source (Garnett and Smethurst 2001, Garnett et al. 2001, 2003). Secondly, the uptake efficiency of waterlogged roots is significantly decreased through root death (Morard and Silvestre 1996, Lizaso et al. 2001). The reduction in the availability of nutrients and root uptake capacity may result in the development of foliar nutrient deficits in waterlogged plants.

Waterlogging induced nutrient deficiency has been reported in a number of crop species (Bacanamwo and Purcell 1999, Lizaso et al. 2001 and Malik et al. 2001), as well as *E. nitens* (Close and Davidson 2003). In addition, the waterlogging tolerance of a species will have an affect on the nutritional response.

Waterlogging-sensitive species exhibit reduced nitrogen uptake and metabolism under waterlogged conditions compared to more tolerant species (Kreuzweiser et al. 2002).

The development of reduced foliar nutrient status was demonstrated in both *E. globulus* and *E. nitens* seedlings after exposure to long-term waterlogging. Significant reductions in foliar nitrogen, phosphorus and potassium were reported after three weeks of hypoxia (Chapter 6); nitrogen, phosphorus, potassium, magnesium and calcium after 10 weeks of waterlogging (Chapter 4) and in phosphorus, potassium and calcium after 28 weeks of waterlogging (Chapter 3). Therefore, the reduction in plant nutrient status under long-term waterlogging was deemed to be the primary cause of the loss in growth and productivity of *E. globulus* and *E. nitens*. As the concentration of nitrogen, phosphorus and potassium is greatest in the upper crown of eucalypts (Grove et al. 1996), the foliar nutrient analysis conducted during this project may in fact, underestimate the whole plant nutrient status.

During long-term waterlogging, the retranslocation of nutrients from old leaves to expanding leaves may be an important mechanism in reducing nutrient deficiency in apical foliage. In eucalypts, a high proportion of nitrogen and phosphorus is remobilised during senescence (Grove et al. 1996). This may explain why the concentration of nitrogen was not significantly reduced in waterlogged plants after 28 weeks (Chapter 3). *Eucalyptus nitens* was observed to have a higher degree of leaf senescence and abscission than *E. globulus* in waterlogged treatments, which may explain why reductions in foliar nutrition of *E. nitens* were not as severe as *E. globulus*. In the field, the process of net retranslocation of nutrients in *E. globulus* is most active during spring and summer (Saur et al. 2000), coinciding with the period of maximum growth.

Foliar nutrient deficiency can be alleviated by the application of nitrogen, applied using various methods. Nitrogen supplements result in the increased uptake of nitrogen, as well as other nutrients, including phosphorus, potassium and zinc (Singh et al. 2002), and result in a positive growth response (Ogden et al. 2002). Fertilisation had a significant effect on waterlogged eucalypts, resulting in increased growth when applied prior to the application of waterlogging (Chapters 3 and 4). It was determined that seedlings with a higher nutrient status were more tolerant to waterlogging, both under long-term conditions and under short-term hypoxia.

When a number of methods of fertiliser application were investigated (Chapter 4), it was found that soil based applications of slow release fertilisers applied during drained periods (either prior to or after a waterlogging event) were the most effective in reducing waterlogging damage. This result is supported by previous work, where the uptake and increase in foliar concentrations of nutrients occurred rapidly after flooding (Lizaso et al. 2001, Slaton et al. 2004). *Eucalyptus globulus* was more responsive to the application of fertiliser than *E. nitens*.

This study examined the relative contribution of reduced soil availability and root uptake of nutrients in the development of foliar nutrient deficiency in *E. globulus* and *E. nitens*. The capacity for hypoxic roots to take up, transport and effectively accumulate nitrogen, phosphorus and potassium was demonstrated in the hydroponic experiment (Chapter 6). Therefore, reduced soil availability of essential plant nutrients, under hypoxic conditions, was deemed to be the primary cause of reduced plant nutrient status developed under waterlogged conditions.

Root aerenchyma provides a low resistance internal pathway for the transport of oxygen and other gases between the shoot and root (Armstrong 1979).

Aerenchymatous root tissue which is developed through cell death also decreases the oxygen demand of the root (Evans 2003). Aerenchymatous roots have also been reported to be as effective as non-aerenchymatous roots, at nutrient uptake (Lizaso et al. 2001), and may reduce the plants demand for nutrients through reduced tissue mass (Fan et al. 2003). Non-aerenchymatous roots lose the

capacity to take up nutrients because of oxygen deficiency and the lack of respiratory substrates for energy-dependent processes. Therefore, the capacity for aerenchymatous root development is a good indicator of the tolerance of a species to waterlogging.

The development of aerenchymatous roots during waterlogging was observed in both species under a range of waterlogging treatments. The capacity for aerenchymatous root development has been reported to be greater in *E. globulus* than *E. nitens* (Connell 1998). *Eucalyptus nitens* developed aerenchyma in primary roots, observed as swollen root tips, after exposure to long periods of waterlogging (28 weeks; see Chapter 3). This capacity for the development of primary root aerenchyma was not observed in *E. globulus*, and indicates the inherently higher tolerance of *E. nitens* to long term waterlogging. This result is not consistent with the conclusions of Connell (1998).

This contradiction of results may be explained by the differences in the experimental technique used between the two studies. In the work of Connell (1998) potting mix was used as the potting medium. In the long term waterlogging experiment conducted during this study (Chapter 3), native soil types were utilised. Differences in root morphology may arise from differences in the soil medium, which may be textural, structural, chemical and nutritive. This may also explain the contradiction of results between Chapters 3 and 4 of this thesis, in terms of aerenchyma development. The use of potting mix (Chapter 4) resulted in the greater development of aerenchymatous roots in *E. globulus* than *E. nitens*, similarly to Connell (1998). Whereas, when grown in native soil types, *E. nitens* had a greater capacity for aerenchymatous root development. Therefore, soil type is a critical factor controlling the development of aerenchymatous roots in these species.

The period of waterlogging treatment is another factor which may explain the contrasting results of Connell (1998) and of those presented here. Waterlogging was applied only for a period of 30 days in Connell (1998), compared to the 28 week and 10 week treatments investigated in Chapters 3 and 4. Field

investigations (presented in Chapter 2), showed that water can remain at the soil surface, for periods in excess of three months. This result highlights the importance of waterlogging during the early establishment phase of eucalypt plantations while seedlings are small, and the need to investigate seedling response to waterlogging, under glasshouse conditions, for long periods (greater than 90 days).

Eucalyptus globulus had a significantly higher mass of aerenchymatous roots than *E. nitens*, but the roots had a lower porosity (Chapter 6). In contrast, *E. nitens* produced few, highly porous aerenchymatous roots. The induction of aerenchymatous root development (see Chapter 5) showed that *E. globulus* produced a relatively large aerenchymatous root system under long-term exposure to hypoxic pre-treatments, while *E. nitens* produced a small amount of highly aerenchymatous roots under all treatments. This led to the conclusion that *E. nitens* is inherently more waterlogging-tolerant than *E. globulus*. However, *E. globulus* demonstrated a greater capacity for increased waterlogging tolerance following application of fertiliser.

A soil-based application of slow-release fertiliser applied during well-drained periods (either prior to or after a waterlogging event) was the most effective in terms of improving the growth and development of morphological adaptations in waterlogged *E. globulus* and *E. nitens*. A significant increase in the foliar concentration of nitrogen, phosphorus and calcium was found under these applications, indicative of the ability of the root system to quickly increase the uptake rate during drained periods. Foliar spray applications of fertiliser were not effective at ameliorating the reduction in foliar nutrient status caused by waterlogging.

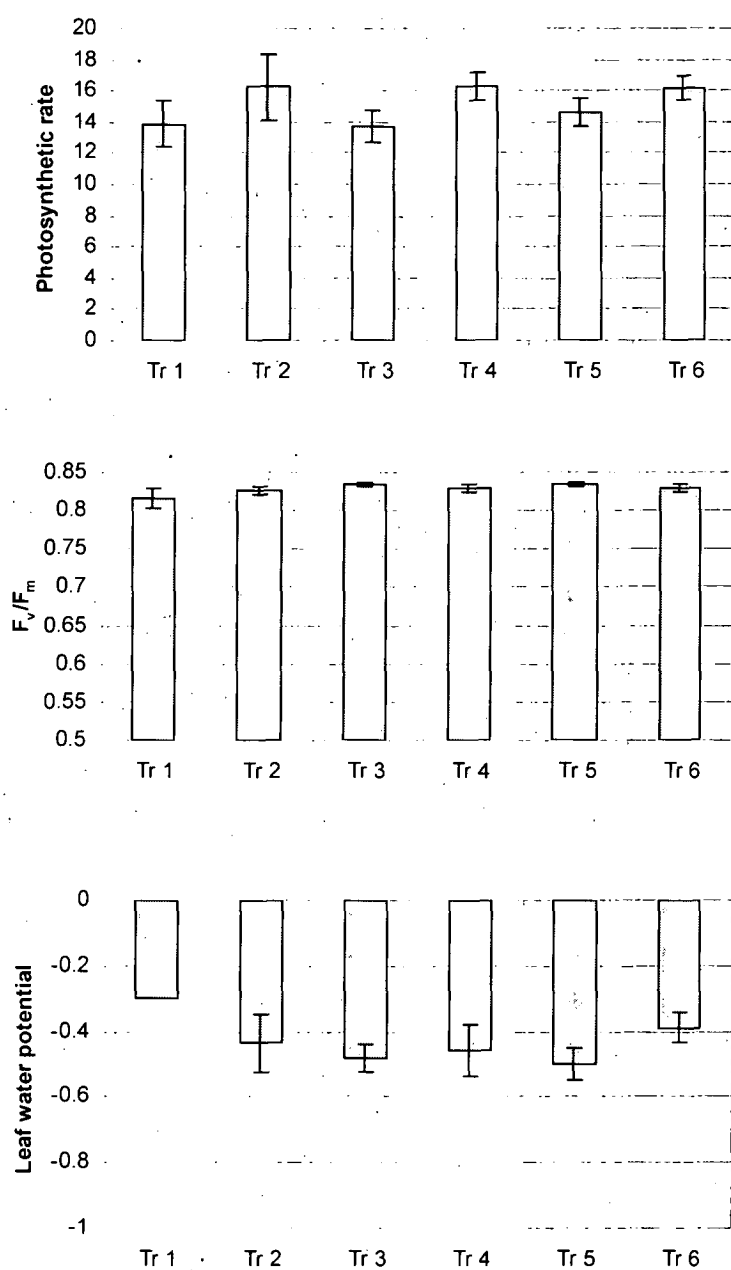
From this study a number of recommendations can be made to improve plantation management in waterlogged sites;

1. The predictable seasonal development of waterlogging, during winter and spring, enables the effective management of waterlogged areas.

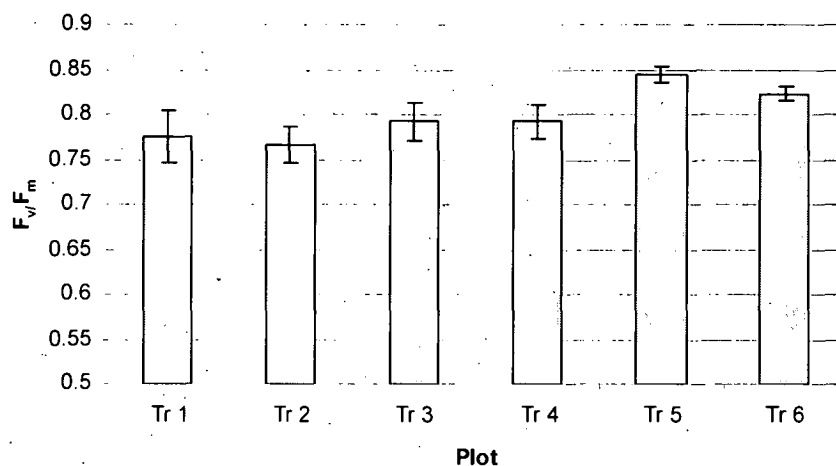
Additional mounding of waterlogging-prone sites prior to planting, may assist in tree roots avoiding the hypoxic rooting zones.

2. Fertilisation of areas likely to become waterlogged, during periods when they are well drained (i.e. summer and autumn) will increase the productivity of waterlogged trees. This management option may also provide a means of expanding the area suitable for plantation establishment.
3. Application of fertiliser to waterlogged trees may only be required during the initial two years of plantation establishment.
4. *Eucalyptus nitens*, as the more waterlogging-tolerant species, should be preferentially planted into sites susceptible to waterlogging, where no additional management techniques are applied. Though at sites where specialised management may be employed, *E. globulus* will be more responsive.

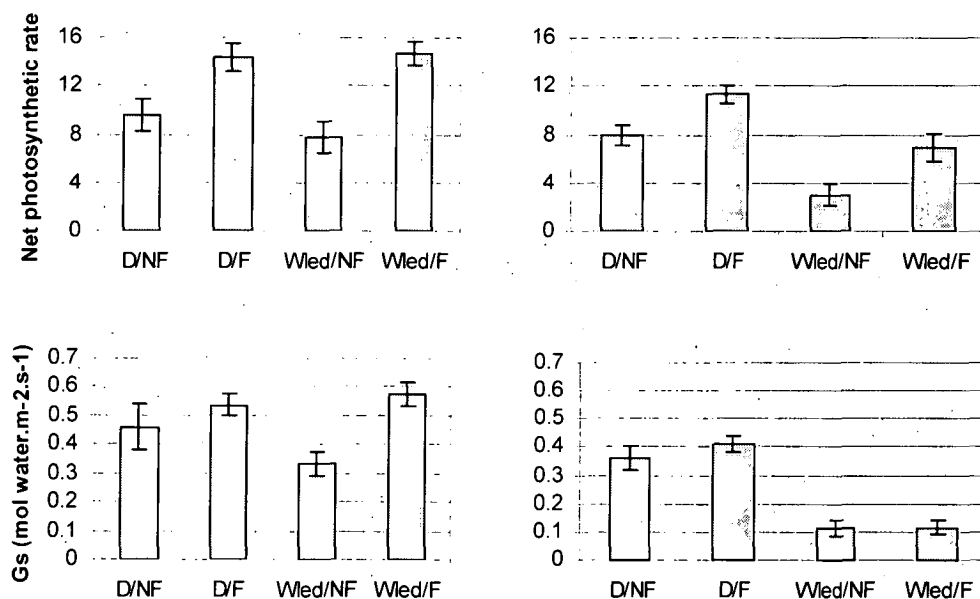
~ APPENDIX ~



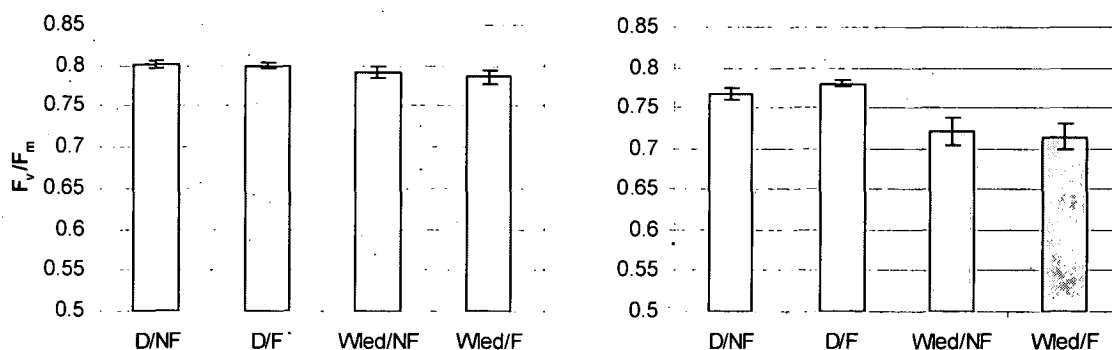
Appendix 1 (a) Midday net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (b) Photosynthetic efficiency (predawn F_v/F_m) and (c) Predawn leaf water potential (MPa) of transect plots Tr1 (waterlogged) through to Tr6 (drained) in December 2003.



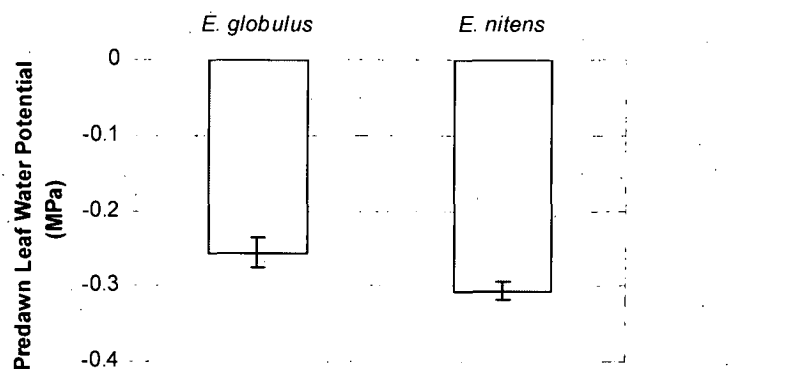
Appendix 2 Photosynthetic efficiency (predawn F_v/F_m) of plots along the transect at area 1 from waterlogged trees (plot Tr 1) to drained trees (plot Tr 6) in May 2004.



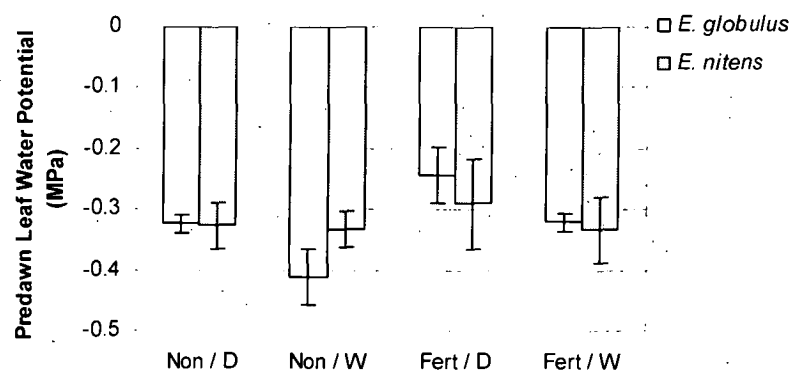
Appendix 3 Net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of drained (D), waterlogged (Wled), non-fertilised (NF) and fertilised (F) *E. nitens* (left) and *E. globulus* (right) seedlings after three weeks of waterlogging.



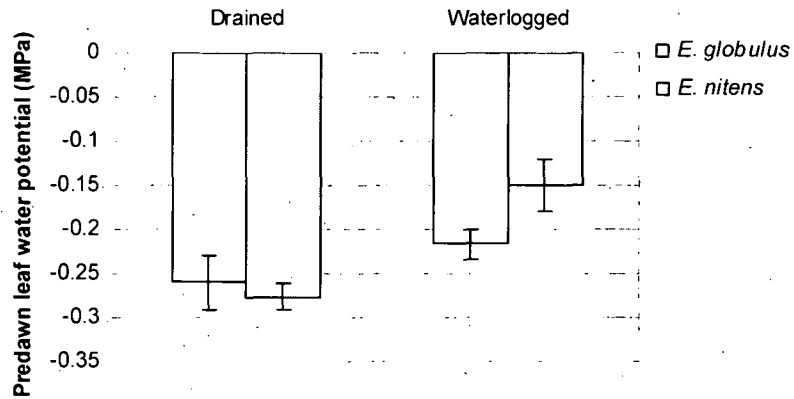
Appendix 4 Photosynthetic efficiency (predawn F_v/F_m) of drained (D), waterlogged (Wled), non-fertilised (NF) and fertilised (F) *E. nitens* (left) and *E. globulus* (right) seedlings after 17 weeks of waterlogging.



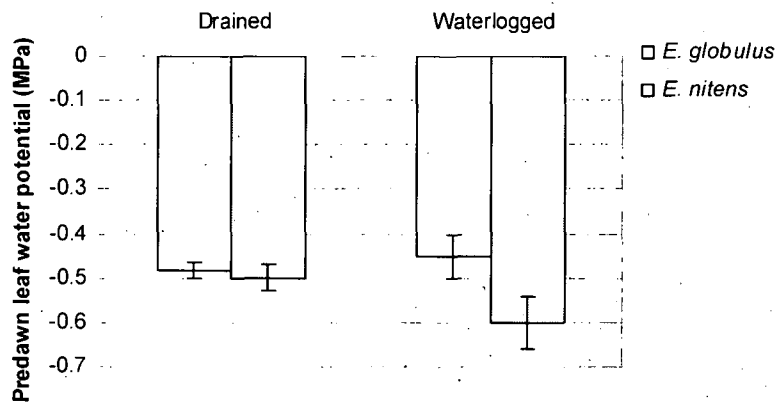
Appendix 5.1 Predawn leaf water potential (MPa) of *E. globulus* and *E. nitens* prior to waterlogging (May 2004).



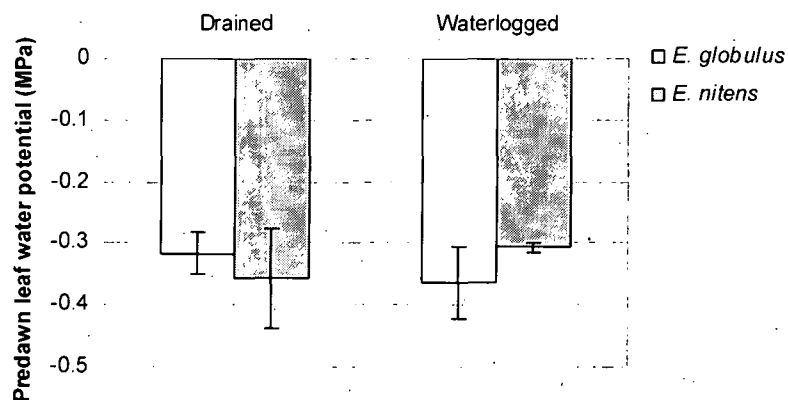
Appendix 5.2 Predawn leaf water potential (MPa) of *E. globulus* and *E. nitens* after three weeks of waterlogging (8 June 2004). Non = non-fertilised, Fert = fertilised, D = drained and W = waterlogged.



Appendix 5.3 Predawn leaf water potential (MPa) of waterlogged and drained *E. globulus* and *E. nitens* after six weeks of waterlogging (29 June 2004).



Appendix 5.4 Predawn leaf water potential (MPa) of waterlogged and drained *E. globulus* and *E. nitens* after ten weeks of waterlogging (July 2004).



Appendix 5.5 Predawn leaf water potential (MPa) of waterlogged and drained *E. globulus* and *E. nitens* after four weeks of recovery (August 2004).

~ LITERATURE CITED ~

Akilan K, Farrell R C C, Bell D T and Marshall J K (1997) Responses of clonal river red gum (*Eucalyptus camaldulensis*) to waterlogging by fresh and salt water. *Australian Journal of Experimental Agriculture* **37** : 243-248.

Andersen P C, Lombard P B and Westwood M N (1984) Effect of root anaerobiosis on the water relations of several *Pyrus* species. *Physiologia Plantarum* **62** : 245-252.

Anderson P H and Pezeshki S R (2001) Effects of flood pre-conditioning on responses of three bottomland tree species to soil waterlogging. *Journal of Plant Physiology* **158** : 227-233.

Andrews D L, Drew M C, Johnson J R and Cobb B G (1994) The response of maize seedlings of different ages to hypoxic and anoxic stress. *Plant Physiology* **105** : 53-60.

Angelov M N, Sung S, Doong R L, Harms W R, Kormanik P P and Black C C (1996) Long and short term flooding effects on survival and sink-source relationships of swamp-adapted tree species. *Tree Physiology* **16** : 477-484.

Armstrong W (1975) Waterlogged soils. In 'Environment and plant ecology'. Ed. Etherington J R. pp 181-218. John Wiley and Sons, London.

Armstrong W (1979) Aeration in higher plants. *Advances in Botanical Research* **7** : 225-332.

Armstrong W, Justin S H F W, Beckett P M and Lythe S (1991) Root adaptation to soil waterlogging. *Aquatic Botany* **39** : 57-73.

Armstrong W (1971) Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration, and waterlogging. *Physiologia Plantarum* **25** : 192-197.

Armstrong W, Healy M T and Lythe S (1983) Oxygen diffusion in pea II. Oxygen concentration in the primary root apex as affected by growth, the production of laterals and radial oxygen loss. *New Phytologist* **94** : 549-559.

Armstrong W, Healy M T and Webb T (1982) Oxygen diffusion in pea I. Pore space resistance in the primary root. *New Phytologist* **91** : 647-659.

Arnon D I (1937) Ammonium nitrate nitrogen nutrition of barley and rice at different seasons in relation to H-ion concentration, manganese, copper and oxygen supplied. *Soil Science* **44** : 91-121.

Ashraf M and Yasmin H (1991) Differential waterlogging tolerances in three grasses of contrasting habitats: *Aeluropus lagopoides* (L.) Trin., *Cyanodon dactylon* (L.) Pers. and *Leptochloa fusca* (L.) Kunth. *Environmental and Experimental Botany* **31** : 437-445.

Atwell B J, Thomson C J, Greenway H, Ward G and Waters I (1985) A study of the impaired growth of roots of *Zea mays* seedlings at low oxygen concentrations. *Plant, Cell and Environment* **8** : 179-188.

Bacanamwo M and Purcell L C (1999) Soybean root morphological and anatomical traits associated with acclimation to waterlogging. *Crop Science* **39** : 143-149.

Baird A J and Wilby A J (1999) Eco-hydrology: Plants and water in terrestrial and aquatic environments xviii + 402 pp. Routledge; London; UK.

Bange M P, Milroy S P and Thongbai P (2004) Growth and yield of cotton in response to waterlogging. *Field Crops Research* **88** : 129-142.

Barling R D, Moore I D and Grayson R B (1994) A quasi-dynamic wetness index for characterizing the spatial distribution of zones of surface saturation and soil water content. *Water Resources Research* **30** : 1029-1044.

Beckman T G, Perry R L, Flore J A (1992) Short term flooding affects gas exchange characteristics of containerised sour cherry trees. *HortScience* **27** (12) : 1297-1301.

Belder P, Bouman B A M, Spiertz J H J, Peng S and Castaneda A R (2005) Crop performance, nitrogen and water use in flooded and aerobic rice. *Plant and Soil* **273** : 167-182.

Berry J and Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* **31** : 491-543.

Blake T J and Reid D M (1981) Ethylene, water relations and tolerance to waterlogging of three *Eucalyptus* species. *Australian Journal of Plant Physiology* **8** : 497-505.

Bishnoi N R and Krishnamoorthy H N (1992) Effect of waterlogging and Gibberellic acid on leaf gas exchange in peanut (*Arachis hypogaea* L.). *Journal of Plant Physiology* **39** : 503-505.

Bohn H L, McNeal B L and O'Connor G A (1979) Oxidation and Reduction In: 'Soil Chemistry' Wiley, New York.

Bouranis D L, Chorianopoulou S N, Siyiannis V F, Protonaris V U and Hawkesford M J (2003) Aerenchyma formation in roots of maize during sulphate starvation. *Planta* **217** (3) : 382-391.

Bradford K J and Shang Fa Yang (1981) Physiological responses of plants to waterlogging. *Hortscience* **16** (1) : 15-30.

Bragina T V, Rodionova N A and Grinieva G M (2003) Ethylene production and activation of hydrolytic enzymes during acclimation of maize seedlings to partial flooding. *Russian Journal of Plant Physiology* **50** (6) : 794-798.

Brisson N, Rebiere B, Zimmer D and Renault P (2002) Response of the root system of a winter wheat crop to waterlogging. *Plant and Soil* **243** : 43-55.

Carreres R, Sendra J, Ballesteros R, Valiente E F, Quesada A, Carrasco D, Leganes F, de la Cuada J G (2003) Assessment of slow release fertilisers and nitrification inhibitors in flooded rice. *Biology and Fertility of Soils* **39**, 80-87.

Chen G P (1989) Alleviation of waterlogging damage to maize plants by the application of nitrogen fertilisers. *Acta Agric. North China* **4** : 26-31.

Chen H J, Qualls R G and Blan R R (2005) Effect of soil flooding on photosynthesis, carbohydrate partitioning and nutrient uptake in the invasive exotic *Lepidium latifolium*. *Aquatic Botany* **82** (4) : 250-268.

Chen H, Qualls R G and Miller G C (2002) Adaptive responses of *Lepidium latifolium* to soil flooding: biomass allocation, adventitious rooting, aerenchyma formation and ethylene production. *Environmental and Experimental Botany* **48** : 119-128.

Cheng L L (2003) Xanthophyll cycle pool size and composition in relation to the nitrogen content of apple leaves. *Journal of Experimental Botany* **54** : 385-393.

Clarkson D T and Hanson J B (1980) The mineral nutrition of higher plants. *Annual Review of Plant Physiology* **31** : 239-298.

Clemens J, Kirk A and Mills P D (1978) Effect of waterlogging and an ethylene-releasing growth substance on *E. robusta*, *E. grandis* and *E. saligna*. *Oecologia* **34** : 125-131.

Clemens J and Pearson C J (1977) The effect of waterlogging on the growth and ethylene content of *Eucalyptus robusta* Sm. (Swamp Mahogany). *Oecologia* **29** : 249-255.

Close D C, Beadle C L and Hovenden M J (2003) Interactive effects of nitrogen and irradiance on sustained xanthophyll cycle engagement in *Eucalyptus nitens* leaves during winter. *Oecologia* **134** : 32-36.

Close D C, Battaglia M, Davidson N J and Beadle C L (2004) Within canopy gradients of nitrogen and photosynthetic activity of *Eucalyptus nitens* and *E. globulus* in response to nitrogen nutrition. *Australian Journal of Botany* **52** (1) : 133-140.

Close D C and Beadle C L (2004) Total, and chemical fractions, of nitrogen and phosphorus in *Eucalyptus* seedling leaves: Effects of species, nursery fertiliser management and transplanting. *Plant and Soil* **259** : 85-95.

Close D C and Davidson N J (2003) Long-term waterlogging: Nutrient, gas exchange, photochemical and pigment characteristics of *Eucalyptus nitens* saplings. *Russian Journal of Plant Physiology* **50** (6) : 843-942.

Close D C, Beadle C L, Brown P H and Holz G K (2000) Cold-induced photoinhibition affects establishment of *Eucalyptus nitens* (Deane and Maiden) Maiden and *Eucalyptus globulus* Labill. *Trees* **15** : 32-41.

Colmer T D, Gibbered M R, Wiengweera A and Tinh T K (1998) The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. *Journal of Experimental Botany* **49** : 1431-1436.

Connell D (1998) The comparative effects of waterlogging on the plantation Eucalypts *E. globulus*, *E. nitens* and *E. regnans*. Honours Thesis, University of Tasmania, Australia.

Crawford R M M (1978) Tolerance of anoxia and the metabolism of ethanol in tree roots. *New Phytologist* **79** : 519-526.

Cresser M, Killham K and Edwards T (1993) Soil Chemistry and its applications. Cambridge University Press, Cambridge.

Davidson N J (1985) Ecophysiological studies of sub-alpine eucalypts. PhD Thesis, University of Tasmania, Australia.

Davidson N J and Reid J (1987) the influence of hardening and waterlogging on the frost resistance of sub-alpine eucalypts. *Australian Journal of Botany* **35** : 91-101.

Davies F S and Flore J A (1986) Flooding, gas exchange and hydraulic conductivity of highbush blueberry. *Physiologia Plantarum* **67** : 545-551.

Davies W J and Kozlowski T T (1974) Stomatal responses of five woody angiosperms to light intensity and humidity. *Canadian Journal of Botany* **52** : 1525-1534.

Demmig B and Bjorkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O₂ evolution in leaves of higher plants. *Planta* **171** : 171-184.

Dell B, Malajczuk N, Xu D and Grove T S (2001) Atlas of Deficiencies. In: Nutrient Disorders in Plantation Eucalypts. 2nd edition. ACIAR Australia Monograph no. 74 vi+188 pages, Canberra.

Drew M C (1983) Plant injury and adaptation to oxygen deficiency in the root environment: A review. *Plant and Soil* **75** : 179-199.

Drew M C (1992) Soil aeration and plant root metabolism. *Soil Science* **154** (4) : 259-268.

Drew M C (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology* **48** : 223-250.

Drew M C and Lynch J M (1980) Soil Anaerobiosis, microorganisms, and root function. *Annual Review Phytopathology* **18** : 37-66.

Drew M C and Siswora E J (1979) The development of waterlogging damage in young barley plants in relation to plant nutrient status and changes in soil properties. *New Phytologist* **82** : 301-314.

Drew M C, Siswora E J and Saker L R (1979) Alleviation of waterlogging damage to young barley plants by application of nitrate and a synthetic cytokinin, and comparison between the effects of waterlogging, nitrogen deficiency, and root excision. *New Phytologist* **82** : 315-329.

Ellis M H and Setter T L (1999) Hypoxia induces anoxia tolerance in completely submerged rice seedlings. *Journal of Plant Physiology* **154** : 219-230.

Engelaar W M H G, Matsumaru T and Yoneyama T (2000) Combined effects of soil waterlogging and compaction on rice (*Oryza sativa* L.) growth, soil aeration, soil N transformations and ^{15}N discrimination. *Biology and Fertility of Soils* **32** : 484-493.

Escamarilla J A and Comerford N B (1998) Phosphorus and potassium depletion by roots of field-grown slash pine: Aerobic and hypoxic conditions. *Forest Ecology and Management* **110** : 25-33.

Evans D E (2003) Aerenchyma formation. *New Phytologist* **161** : 35-49.

Fan M, Zhu J, Richards C, Brown KM and Lynch JP (2003) Physiological roles for aerenchyma in phosphorus - stressed roots. *Functional Plant Biology* **30** : 493-506.

Fenwick I M and Knapp B J (1982) Soils, process and response. Gerald Duckworth and Co. Ltd, London.

Florence R G (1996) Ecology and silviculture of eucalypt forests. CSIRO, Australia.

Foyer C H, Lelandais M and Kunert K J (1994) Photooxidative stress in plants. *Physiologia Plantarum* **92** : 696-717.

Galloway R and Davidson N J (1993) The response of *Atriplex amnicola* to the interactive effects of salinity and hypoxia. *Journal of Experimental Botany* **44** : 653-663.

Garnett T P, Shabala S N, Smethurst P J and Newman I A (2001) Simultaneous measurement of ammonium, nitrate and proton fluxes along the length of eucalypt roots. *Plant and Soil* **236** : 55-62.

Garnett T P, Shabala S N, Smethurst P J and Newman I A (2003) Kinetics of ammonium and nitrate uptake by eucalypt roots and associated proton fluxes measured using ion selective microelectrodes. *Functional Plant Biology* **30** : 1165-1176.

Garnett T P and Smethurst P J (1999) Ammonium and nitrate uptake by *Eucalyptus nitens*: effects of pH and temperature. *Plant and Soil* **214** : 133-140.

Garthwaite A J, von Bothmer R and Colmer T D (2003) Diversity in root aeration traits associated with waterlogging tolerance in the genus *Hordeum*. *Functional Plant Biology* **30** : 875-889.

Germain V, Ricard B, Raymond P and Saglio P H (1997) The role of sugars, hexokinases, and sucrose synthase in the determination of hypoxically induced tolerance to anoxia in tomato roots. *Plant Physiology* **114** : 167-175.

- Ghanem S A, Ali R A, Rezk M M and Abd Ekl-Razik A M (1998) Influence of seasonal flooding period and the application of zinc, iron and manganese on the availability of applied phosphorus, growth and yield of rice. *Egyptian Journal of Agricultural Research* **76** (1) : 175-190.
- Gibberd M R, Colmer T D and Cocks P S (1999) Root porosity and oxygen movement in waterlogging-tolerant *Trifolium tomentosum* and -intolerant *Trifolium glomeratum*. *Plant, Cell and Environment* **22** : 1161-1168.
- Gibberd M R, Gray J D, Cocks P S and Colmer T D (2001) Waterlogging tolerance among a diverse range of *Trifolium* accessions is related to root porosity, lateral root formation and 'aerotropic rooting'. *Annals of Botany* **88** : 579-589.
- Gibbs J, Greenway H (2003) Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Functional Plant Biology* **30** : 1-47.
- Gibbs J, Turner D, Armstrong W, Darwent M J and Greenway H (1998) Response to oxygen deficiency in primary maize roots. I Development of oxygen deficiency in the stele reduces radial solute transport to the xylem. *Australian Journal of Plant Physiology* **25** : 745-758.
- Gill R A and Jackson R B (2000) Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* **147** : 13-31.
- Gilmore A M and Yamamoto H Y (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C18 high-performance liquid chromatographic column. *Journal of Chromatography* **543** : 137-145.
- Grichko V P and Glick B R (2001) Ethylene and flooding stress in plants. *Plant Physiology and Biochemistry* **39** : 1-9.

- Grove T S, Thomson B D and Malajczuk N (1996) Nutritional physiology of eucalypts: uptake, distribution and utilization. In: Nutrition of eucalypts. Attiwill PM and Adams MA (Eds). CSIRO publishing, Melbourne, Australia. PJ
- Hawkins B and Polgase (2000) Foliar concentrations and resorption of nitrogen and phosphorus in 15 species of eucalypt grown under non-limited water and nutrient availability. *Australian Journal of Botany* **48** : 597-602.
- Hill J, Attiwill P M, Uren N C and O'Brien N D (2001) Does manganese play a role in the distribution of the eucalypts? *Australian Journal of Botany* **49** : 1-8.
- Hillil D (1998) Environmental soil physics. Academic Press, USA.
- Honeysett J L, White D A, Worledge D and Beadle C L (1996) Growth and water use of *Eucalyptus globulus* and *E. nitens* in irrigated and rainfed plantations. *Australian Forestry* **59** (2) : 64-73.
- Hortensteiner S and Feller U (2002) Nitrogen metabolism and remobilisation during senescence. 6th International Symposium on Inorganic Nitrogen Assimilation. Reims, France, 8-12 July 2001. *Journal of Experimental Botany* **53** (370) : 927-937.
- Hooda N and Weston C J (1999) Influence of site and fertiliser addition on nutrient cycling in *Eucalyptus globulus* plantations in Gippsland, south-eastern Australia. I. Foliage and litter quality. *Australian Journal of Botany* **47** : 189-206.
- Hook D D, DeBell D S, McKee W H and Askew J L (1983) Responses of loblolly pine (mesophyte) and swamp tupelo (hydrophyte) seedlings to soil flooding and Phosphorus. *Plant and Soil* **71** : 387-394.

- Huang Z A, Jiang D A, Yang Y, Sun J W and Jin S H (2004) Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica* **42** (3) : 357-364.
- Huang B, Johnson J W, Nesmith S and Bridges D C (1994) Growth, physiological and anatomical responses of two wheat genotypes to waterlogging and nutrient supply. *Journal of Experimental Botany* **45** (271) : 193-202.
- Jackson M B (1979) Rapid injury to peas by soil waterlogging. *Journal of the Science of Food and Agriculture* **30** : 143-152.
- Jackson M B (2002) Long-distance signaling from roots to shoots assessed: the flooding story. *Journal of Experimental Botany* **53** (367) : 175-181.
- Jackson M B and Drew M C (1984) Effects of flooding on growth and metabolism of herbaceous plants. In: 'Flooding and plant growth'. Kozlowski T T (Ed) Academic Press Inc.
- Jackson M B, Saker L R, Crisp C M, Else M A and Janowiak F (2003) Ionic and pH signaling from roots to shoots of flooded tomato plants in relation to stomatal closure. *Plant and Soil* **253** : 103-113.
- Janiesch P (1991) Ecophysiological adaptations of higher plants in natural communities to waterlogging. In: 'Ecological responses to environmental stresses'. Rozema J and Verleij J A C (Eds). Kluwer Academic Publishers, Dordrecht.
- Janssens I A, Sampson D A, Curiel-Yuste J, Carrara A and Ceulemans R (2002) The carbon cost of fine root turnover in a Scots pine forest. *Forest Ecology and Management* **168** : 231-240.
- Johnson J, Cobb B G and Drew M C (1989) Hypoxic induction of anoxia tolerance in root tips of *Zea mays*. *Plant Physiology* **91** : 837-841.

Johnson J, Cobb B G and Drew M C (1994) Hypoxic induction of anoxia tolerance in roots of Adh 1 null *Zea mays*. *Plant Physiology* **105** (1) : 61-67.

Justin S H F W and Armstrong W (1987) Anatomy of roots in response to flooding. *New Phytologist* 465-495.

Kaelke C M and Dawson J O (2003) Seasonal flooding regimes influence survival, nitrogen fixation, and the partitioning of nitrogen and biomass in *Alnus incana* ssp. *Rugosa*. *Plant and Soil* **254** : 167-177.

Kato-Noguchi H (2000) Abscissic acid and hypoxic induction of anoxia tolerance in roots of lettuce seedlings. *Journal of Experimental Botany* **51** (352) : 1939-1944.

Kawase M (1981) Anatomical and morphological adaptation of plants to waterlogging. *Hortscience* **16** (1) : 30-34.

Kawase M and Whitmoyer R E (1980) Aerenchyma development in waterlogged plants. *American Journal of Botany* **67**(1) : 30-34.

Kirschbaum M U F and Tompkins D (1990) Photosynthetic response to phosphorus nutrition in *Eucalyptus grandii* seedlings. *Australian Journal of Plant Physiology* **17** : 527-235.

Kozlowski T T (1979) Water supply and tree growth. In "Tree growth and environmental stresses". University of Washington Press, Seattle.

Kozlowski T T (1984) Plant responses to flooding of soil. *Bioscience* **34**(3) : 162-167.

Kozlowski T T (1997) Responses of woody plants to flooding and salinity. *Tree Physiology Monograph No 1*.

Kozłowski T T and Pallardy S G (1979) Stomatal responses of *Fraxinus pennsylvanica* seedlings during and after flooding. *Physiologia Plantarum* **46** : 155-158.

Kramer P J and Jackson W T (1954) Causes of injury to flooded tobacco plants. *Plant Physiology* **29** : 241-245.

Kreuzwieser J, Furniss S and Rennenberg H (2002) Impact of waterlogging on the nitrogen metabolism of flood tolerant and non-tolerant tree species. *Plant, Cell and Environment* **25** : 1039-1049.

Krizek D T (1982) Plant response to atmospheric stress caused by waterlogging. In 'Breeding plants for less favourable environments'. (Eds. Mn Christiansen, CF Lewis, H Lambers) pp. 293-335. (John Wiley and Sons, Inc., New York).

Ladiges P Y and Kelso A (1977) The comparative effects of waterlogging on two populations of *Eucalyptus viminalis* Labill. and one population of *E. ovata* Labill. *Australian Journal of Botany* **25** : 159-169.

Laffan M (1999) report on Field Visit to discuss plantation potential of state forest between Ferny Hill Road and western Boundary of 'Loloma', Bass District. Forestry Tasmania.

Laffan M (2002) Field procedures for recording land attributes and assessing plantation potential in Tasmanian state forest Draft no. 12. Forestry Tasmania.

Laffan M, Grant J and Hill R (1994) How to select highly productive and suitable land for plantations in Tasmania using soil and site properties. In: 'Faces of Farm Forestry', pp. 95-100. Proceedings of the Australian Forest Growers Conference, 2-5 May, Launceston, Tasmania.

Laffan M, Grant J C and Hill R B (1995) Soils of Tasmanian State forests. 1. Pipers Sheet. Soils Bulletin No. 1 Forestry Tasmania.

- Lizaso JI, Melendez LM, Raminez R (2001) Early flooding of two cultivars of tropical maize. II. Nutritional responses. *Journal of Plant Nutrition* **24**(7) : 997-1011.
- Long S P, Humphries S and Falkowski P G (1994) Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology* **45** : 633-662.
- Lowther J R (1980) Use of a single acid – hydrogen peroxide digest for the analysis of *Pinus radiata* needles. *Communications in Soil Science and Plant Analysis* **11** : 175-188.
- Lu C M, Lu Q T, Zhang J H and Kuang T Y (2001) Characterisation of photosynthetic pigment composition, PS II photochemistry and thermal energy dissipation during leaf senescence of wheat plants grown in the field. *Journal of Experimental Botany* **52** : 1805-1810.
- Malik A I, Colmer T D, Lambers H and Schortemeyer M (2001) Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Australian Journal of Plant Physiology* **28** : 1121-1131.
- Malik A I, Colmer T D, Lambers H and Schortemeyer M (2002) Short-term waterlogging has long-term effects on the growth and physiology of wheat. *New Phytologist* **153** : 225-236.
- Marcar N E (1993) Waterlogging modifies growth, water use and ion concentrations in seedlings of salt-treated *Eucalyptus camaldulensis*, *E. tereticornis*, *E. robusta* and *E. globulus*. *Australian Journal of Plant Physiology* **20**: 1-13.
- Marcar N E, Crawford D F, Saunders A, Matheson A C and Arnold R A (2002) Genetic variation among and within provenances and families of *Eucalyptus*

grandis W. Hill and *E. globulus* Labill. Subsp. *globulus* seedlings in response to salinity and waterlogging. *Forest Ecology and Management* **162** : 231-249.

Marschner H (1995) Mineral Nutrition of higher plants 2nd Edition. Academic Press, London.

Marschner H, Kirkby E A and Cakmak I (1996) Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *Journal of Experimental Botany* **47** : 1255-1263.

McFarlane D and Wheaton B (1990) The extent and cost of waterlogging. *WA Journal of Agriculture* **31** : 44-47.

McLaren R G and Cameron K C (1996) Soil Science 2nd edition. Oxford University Press, Australia.

Melhuish F M, Humphreys E, Muirhead W A and White R J H (1991) Flood irrigation of wheat on a transitional red-brown earth. I. Effect of duration of ponding on soil water, plant growth, yield and N uptake. *Australian Journal of Agricultural Research* **42** : 1023-1035.

Morard P and Silvestre J (1996) Plant injury due to oxygen deficiency in the root environment of soil-less culture: a review. *Plant and Soil* **184** : 243-254.

Morales L A, Vazquez S, Paz-Gonzalez A (2002) Nutrient status on an acid soil as affected by lime amendments after flooding. *Communications in Soil Science and Plant Analysis* **33** (15-18) : 3007-3014.

Moroni M T, Worledge D and Beadle C L (2003) Root distribution of *Eucalyptus nitens* and *E. globulus* in irrigated and droughted soil. *Forest Ecology and Management* **177** : 399-407.

- Mummery D Battaglia M Beadle C L Turnbull C R A and McLeod R (1999) An application of terrain and environmental modeling in a large scale forestry experiment. *Forest Ecology and Management* **118** : 149-159.
- Mummery D and Battaglia M (2001) Applying ProMod Spatially across Tasmania with sensitivity analysis to screen for prospective *Eucalyptus globulus* plantation sites. *Forest Ecology and Management* **140** : 51-63.
- National Forest Inventory 2004, *National Plantation Inventory Update – March 2004*, Bureau of Rural Sciences, Canberra, Australia.
- Nicolas E, Torrecillas A, Dell’Amico J and Alarcon J J (2005) The effect of short-term flooding on the sap flow, gas exchange and hydraulic conductivity of young apricot trees. *Trees* **19** : 51-57.
- Nicolls K D, Honeysett J L and Graley A M (1982) Soil storage of water under eucalypt forest in S.E Tasmania. *In proc. First National Symp. of Forest Hydrology*. E M O’Loughlin and L J Bren (Eds). Pp 39-42 Institute of Engineers, Canberra.
- Noble I R (1989) Ecological traits of the *Eucalyptus* L’Herit. Subgenera *Monocalyptus* and *Symphyomyrtus*. *Australian Journal of Botany* **37** : 207-224.
- Ogden R W, Thoms M C and Levings P L (2002) Nutrient limitation of plant growth on the floodplain of the Narran River, Australia: growth experiments and a pilot soil survey. *Hydrobiologia* **489** : 277-285.
- Orme K, Orr S, Gerrand A and Todd G (1992) Potential of low fertility sites for plantations in north-eastern Tasmania. *Tasforests* : 69-75.
- Pang J, Zhou M, Mendham N and Shabala S (2004) Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. *Australian Journal of Agricultural Research* **55** : 895-906.

- Pasrija A K and Chithara S D (1988) Nutrient uptake, chlorophyll content and leaf xylem water potential as affected by waterlogging in different stionic combinations of peach and plum. *Progressive Horticulture* **20** : 109-113.
- Patrick W H and Jugsujinda A (1992) Soil chemistry. *Soil Science Society of America Journal* **56** : 1071-1073.
- Patrick W H and Mahapatra I C (1968) Transformation and availability to rice of nitrogen and phosphorus in waterlogged soils. *Advances in Agronomy* **20** : 323-359.
- Pereira J S and Kozlowski T T (1977) Variations among woody angiosperms in response to flooding. *Physiologia Plantarum* **41** : 184-192.
- Pezeshki S R (1993) Differences in patterns of photosynthetic responses to hypoxia in flood-tolerant and flood-sensitive tree species. *Photosynthetica* **28** (3) : 423-430.
- Pezeshki S R and Chambers (1984) Stomatal and photosynthetic responses of sweet gum (*Liquidambar styraciflua*) to flooding. *Canadian Journal of Forest Research* **15** : 371-375.
- Pezeshki S R, Pardue J H and DeLaune R D (1996) Leaf gas exchange and growth of flood-tolerant and flood-sensitive tree species under low soil redox conditions. *Tree Physiology* **16** : 453-458.
- Phillips I R (1998) Phosphorus availability and sorption under alternating waterlogged and drying conditions. *Communications in Soil Science and Plant Analysis* **29** (19 and 20) : 3045-3059.
- Phillips I R (1999) Nitrogen availability and sorption under alternating waterlogged and drying conditions. *Communications in Soil science and Plant Analysis* **30** (1 and 2) : 1-20.

Phillips I R (2001) Nitrogen and phosphorus transport in soil using simulated waterlogged conditions. *Communications in Soil Science and Plant Analysis* **32** (5 and 6) : 821-842.

Phillips I R and Greenway M (1998) Changes in water-soluble and exchangeable ions, cation exchange capacity, and phosphorus_{max} in soils under alternating waterlogged and drying conditions. *Communications in Soil Science and Plant Analysis* **29** (1 and 2) : 51-65.

Plesnicar M, Kastori R, Petrovic N and Pankovic D (1994) Photosynthesis and chlorophyll florescence in sunflower (*Helianthus annuus* L.) leaves as affected by phosphorus nutrition. *Journal of Experimental Botany* **45** : 919-924.

Ponnamperuma F N (1972) The chemistry of submerged soils. *Advances in Agronomy* **24** : 29-96.

Poot P and Lambers H (2003) Growth responses to waterlogging and drainage of woody *Hakea* (Proteaceae) seedlings, originating from contrasting habitats in south-western Australia. *Plant and Soil* **253** : 57-70.

Pryor R, Davidson NJ, Close DC (2005). Waterlogging duration: an inter-specific comparison of *Leptospermum scoparium* (Forst), *Acacia melanoxylon* (R.Br.), *Nothofagus cunninghamii* (Hook.) and *Eucalyptus obliqua* (L'Herit). *Austral Ecology*. (In press).

Raskin I (1983) A method for measuring leaf volume, density, thickness, and internal gas volume. *HortScience* **18** : 698-699.

Reece C F and Riha S J (1991) Role of root systems of eastern larch and white spruce in response to flooding. *Plant, Cell and Environment* **14** : 229-234.

Resh S R, Battaglia M, Worledge D and Ladiges S (2003) Coarse root biomass for eucalypt plantations in Tasmania, Australia: Sources of variation and methods for assessment. *Trees: Structure and Function* **17** (5) : 389-399.

SAS Institute (2001) SAS/STAT user's guide for [personal computers, release 9.1 (SAS Institute Inc. Cary, New York).

Saur E, Nambiar E K S and Fife D N (2000) Foliar nutrient retranslocation in *Eucalyptus globulus*. *Tree Physiology* **20** : 1105-1112.

Schmull M and Thomas F M (2000) Morphological and physiological reactions of young deciduous trees (*Quercus robur* L., *Q. petraea* (Matt.) Liebl., *Fagus sylvatica* L.) to waterlogging. *Plant and Soil* **225** : 227-242.

Scholander P F, Hammel H T, Bradstreet E D and Hemmingsen E A (1965) Sap pressure in vascular plants. *Science* **148** : 339-345.

Sena Gomes A R and Kozlowski T T (1980) Effects of flooding on *Eucalyptus camaldulensis* and *E. globulus* seedlings. *Oecologia* **46** : 139-142.

Sharma D P and Swarup A (1988) Effects of short-term flooding on growth, yield and mineral composition of wheat on sodic soil under field conditions. *Plant and Soil* **107** : 137-143.

Singh Y V, Swamp A, Gupta S K (2002) Alleviating adverse effects of waterlogging through top-dressed Urea-N on growth, yield and mineral composition of sorghum in a sodic soil. *Agrochimica* **96** (3-4) : 89-99.

Slaton N A, Dunn D and Pugh B (2004) Potassium nutrition of flood-irrigated rice. *Better Crops with Plant Food* **88** (3) : 20-22.

Smirnoff N and Crawford R M M (1983) Variation in the structure and response to flooding of root aerenchyma in some wetland plants. *Annals of Botany* **51** : 237-249.

Sojka R E and Stolzy L H (1980) Soil – oxygen effects on stomatal response. *Soil Science* **130** (6) : 350-358.

Sun O J, Sweet G B, Whitehead D and Buchan G D (1995) Physiological responses to water stress and waterlogging in *Nothofagus* species. *Tree Physiology* **15** : 629-638.

Swarup A and Sharma D P (1993) Influence of top-dressed nitrogen in alleviating adverse effects of flooding on growth and yield of wheat in a sodic soil. *Field Crops Research* **35** : 93

Tang Z C and Kozlowski T T (1984) Ethylene production and morphological adaptation of woody plants to flooding. *Canadian Journal of Botany* **62** : 1659-1664.

Thomson C J, Armstrong W, Greenway H and Waters I (1992) Tolerance of wheat (*Triticum aestivum* cvs Gamanya and Kite) and triticale (*Triticosecale* cv. Muir) to waterlogging. *New Phytologist* **120** : 335-344.

Thomson C J, Armstrong W, Waters I and Greenway H (1990) Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. *Plant, Cell and Environment* **13** : 395-403

Topa M A and Cheeseman J M (1992) Effects of root hypoxia and a low Phosphorus supply on relative growth, carbon dioxide exchange rates and carbon partitioning in *Pinus serotina* seedlings. *Physiologia Plantarum* **86** : 136-144.

Trought M C T and Drew M C (1980) The development of waterlogging damage in wheat seedlings (*Triticum aestivatum* L.) 1. Shoot and root growth in relation to

changes in the concentrations of dissolved gases and solutes in the soil solution. *Plant and Soil* **54** : 77-94.

Turnbull C R A, McLeod D E., Beadle C L and Ratowsky D A (1993) Comparative early growth of *Eucalyptus* species of the subgenera Monocalyptus and Symphyomyrtus in intensively-managed plantations in southern Tasmania. *Australian Forestry* **56** (3) : 276-286.

Umaharan P, Ariyanayagam R P and Haque S Q (1997) Effect of short-term waterlogging applied at various growth phases on growth, development and yield in *Vigna unguiculata*. *Journal of Agricultural Science* **128** : 189-198.

Van den Berg G A and Loch J P G (2000) Decalcification of soils subject to periodic waterlogging. *European Journal of Soil Science* **51** (91) : 27-33.

Van der Moezel P G, Watson L E, Pearce-Pinto G V N and Bell D T (1988) The response of six *Eucalyptus* species and *Casuarina obesa* to the combined effect of salinity and waterlogging. *Australian Journal of Plant Physiology* **15** (3) : 465-474.

Vartapetian B B, Andreeva I N, Generozova I P, Polyakova L I, Maslovas I P, Dolgikh Y I and Stepanova A Y (2003) Functional electron microscopy in studies of plant response and adaptation to anaerobic stress. *Annals of Botany* **91** : 155-172.

Vartpaetian B B and Jackson M B (1997) Plant adaptations to anaerobic stress. *Annals of Botany* **79** (Supplement A) : 3-20.

Verhoeven A S, Demmig-Adams B and Adams W W II (1997) Enhanced employment of the xanthophylls cycle and thermal energy dissipation in spinach exposed to high light and nitrogen stress. *Plant Physiology* **113** : 817-824.

Visser E J W, Bogemann G M and Blom C W P M (1996) Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots. *Journal of Experimental Botany* **47** : 403-410.

Visser E J W, Voesenek L A C J, Vartapetian B B and Jackson M B (2003) Flooding and plant growth. *Annals of Botany* **91** : 107-109.

Voesenek L A C J, Armstrong W, Bogemann G M, McDonald M P and Colmer T D (1999) A lack of aerenchyma and high rates of radial oxygen loss from the root base contribute to the waterlogging intolerance of *Brassica napus*. *Australian Journal of Plant Physiology* **26** : 87-95.

Voesenek L A C J, Van der Sman A J M, Harren F J M and Blom C W P M (1992) An amalgamation between hormone physiology and plant ecology: a review on flooding resistance and ethylene. *Journal of Plant Growth Regulation* **11** : 171-188.

Waters I, Morrell S, Greenway H and Colmer T D (1991) Effects of anoxia on wheat seedlings. *Journal of Experimental Botany* **42** (244) : 1437-1447.

Wetson E R, Lapins P and Barren R J W (1976) Effect of waterlogging on the growth, grain and straw yield of wheat, barley and oats. *Australian Journal of Experimental Animal Husbandry* **16** : 114

Wigmosta M S, Vail L W and Lettenmaier D P (1994) A distributed hydrology-vegetation model for complex terrain. *Water Resources Research* **30** (6) : 1665-1679.

Worledge D, Honeysett J L, White D A, Beadle C L and Hetherington S J (1998) Scheduling irrigation in plantations of *E. globulus* and *E. nitens*: A practical guide. *Tasforests* **10** : 91-101.

Yang C, Yang L, Yang Y and Quyang Z (2004) Rice root growth and nutrient uptake as influenced by organic manure in continuously and alternately flooded paddy soils. *Agricultural Water Management* **70** (1) : 67-81.

Yordanova R Y, Uzunova A N and Popova L P (2005) Effects of short-term soil flooding on stomata behaviour and leaf gas exchange in barley plants. *Biologia Plantarum* **49** (2) : 317-319.

Zhang J and Davies W J (1986) Chemical and hydraulic influences on the stomata of flooded plants. *Journal of Experimental Botany* **37** (183) : 1479-1491.

Zhang Q and Greenway H (1994) Anoxia tolerance and anaerobic catabolism of aged beetroot storage tissue. *Journal of Experimental Botany* **45** : 567-575.

Zhou W, Zhao D, Lin X (1997) Effects of waterlogging on N accumulation and alleviation of waterlogging damage by application of N fertiliser and Mixatalol in winter rape (*Brassica napus* L.). *Journal of Plant Growth Regulation* **16** : 47-53.