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**Promoting seedling physiological performance and early establishment in degraded
Mediterranean-type ecosystems**

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Abstract Mediterranean climate ecosystems (MCEs) are amongst the most heavily degraded ecosystems worldwide. Restoration efforts are challenged by high vulnerability to extreme drought, which is projected to become more frequent with future climate change. The aim of our study was to determine whether restoration efforts could be enhanced through the individual and combined effects of the site preparation technique of soil ripping and the addition of fertilisers. We tested the effects of ripping and fertiliser (\pm surfactant) on survival, shoot height, crown health, root biomass and leaf physiology of *Eucalyptus gomphocephala* seedlings in degraded MCEs in Western Australia. Restoration treatments had a much stronger impact under closed canopy (forest) compared to open canopy (woodland) conditions. In the forest, soil ripping doubled seedling survival and together with fertiliser application enhanced shoot height 2.35-fold relative to control seedlings. Ripping resulted in more favourable leaf water potentials and enhanced stomatal conductance suggesting increased water availability compared to unripped soil. In the woodland, fertilisation improved seedling survival and stimulated shoot height (+45%) and root biomass (>2-fold). Our results demonstrate that restoration techniques targeting the co-limitation by water and nutrients can greatly increase chances of successful restoration in these types of ecosystems.

Keywords Revegetation; Nutrients; Fertilizer; Surfactant; Restoration; rehabilitation

Introduction

Ecosystem fragmentation via extensive clearing for agriculture and urbanisation and degradation has left few large Mediterranean type climate ecosystem (MCE) forests and woodlands intact (Laurance et al. 2011; Lindner et al. 2009). In addition, recent drought and heat events associated

with climate change have led to massive collapses and altered ecosystem processes in many of these systems (Allen et al. 2010; Dimitrakopoulos et al. 2011; Matusick et al. 2013; Matusick et al. 2012). Climate change is projected to impact MCEs significantly in the future (Yates et al. 2010) with Klausmeyer and Shaw (2009) predicting that Australian MCEs will contract to only 77-49% of their current size. Clearly, these threats have severe implications for ecosystem structure and functioning and jeopardise the ability to support the high levels of biodiversity MCEs are renowned for.

To increase the integrity and resilience of MCE ecosystems, arrest and reverse degradation, and to buffer systems from the wide range of threats, ecological restoration is essential at unprecedented scales (Hobbs and Norton 1996; Manning et al. 2006). This urgently requires the development of superior and appropriate techniques for successful and cost effective restoration at a landscape scale, using minimal amounts of valuable propagules and resources such as moisture and nutrients (Ruthrof et al. 2013b). The use of seedlings, rather than seeds, is often favoured in restoration of degraded MCEs due to higher survivorship rates (Close et al. 2009; Thea et al. 2011). However, the survival of planted seedlings can be hindered by low moisture availability during the summer drought period (Verdaguer et al. 2011). Indeed, the first summer following planting is regarded as the most critical in terms of survivorship in MCEs (Gomez-Aparicio et al. 2004; Lloret et al. 1999; Tsakalidimi et al. 2005). The projected drying climate for many MCEs will make successful restoration increasingly difficult in the future (de Dios et al. 2007; Klausmeyer and Shaw 2009).

Measuring early seedling establishment success is crucial for identifying potentially beneficial restoration treatments. Seedling mortality can be due to a variety of reasons, including water deficit, high temperatures, animal browsing, competition, and pre and post-emergent diseases (Benayas 1998; Chaar et al. 2008; Close et al. 2009). Therefore, restoration tools aiming at maximising above and below ground seedling vigour are critical for drought recovery, competing for resources with other plants (including invasive species), pathogen resistance and resilience against herbivores (Ruthrof et al. 2010). In this context, physiological measurements are becoming increasingly important for restoration ecologists because they allow assessment of more subtle seedling responses to restoration treatments and may thus help guide the development of modern restoration strategies (Close et al. 2009; Rood et al. 2003; Vilagrosa et al. 2005). Given the protracted summer drought conditions typical for MCEs, physiological mechanisms for resisting drought are critical for coping with low soil water availability and high evaporative demand (Close et al. 2009) and survival of developing juvenile vegetation (Crombie 1997). Physiological measurements can be used to monitor direct responses to restoration treatments and hence provide a powerful tool for the evaluation of restoration efforts (Cooke and Suski 2008). Leaf gas-exchange and leaf water potentials provide sensitive indicators of changes in plant water and carbon relations associated with restoration treatments (Chen et al. 2005).

Restoration of biotic soil properties is a vital part of long-term ecological restoration. Soil respiration, for example, can be used as an integrative measure of soil biological activity (Kaye and Hart 1998) and provide useful information about below ground responses (from soil microorganisms and plant roots) to restoration techniques as well as the overall impacts of vegetation succession on soil properties (An et al. 2009). Given this, there is growing interest in

soil respiration as one of the indicators of ecological change following restoration (An et al. 2009; Fereidooni et al. 2013; Harris 2003).

Here, we have used the *Eucalyptus gomphocephala* (tuart) ecosystem as a model system to test treatments targeting the severe co-limitation of water and nutrients for restoring severely degraded MCEs. We explored the responses of six-month-old seedlings and soil biological activity (through respiration) to restoration techniques (site preparation, and fertiliser addition) in two degraded MCE communities in southwestern Australia. These sites were degraded over many decades by grazing and weed invasion, but are currently National Parks that still retain adult *E. gomphocephala* trees and have secure land tenure necessary for longer-term studies. Early seedling survival and vigour can be achieved with site preparation techniques such as ripping in MCEs (Barbera et al. 2005; Bocio et al. 2004; Palacios et al. 2009; Vallejo et al. 2006) to alleviate compaction of soils, increase water infiltration (Yates et al. 2000), reduce bulk density and facilitate rapid seedling root growth deeper into the profile during early establishment (Ruthrof et al. 2013a). In deep, sandy, and hydrophobic soils such as those found in sandy plains of southwestern Australia (Roberts and Carbon 1972), soil moisture tends to occur in uneven patterns within the soil profile (Doerr et al. 2000; Letey 2001). However, surfactants or wetting agents can enhance infiltration and improve water availability in hydrophobic soils (Madsen et al. 2012b). The addition of fertilisers has been effective in increasing seedling height in a restoration context (Ruthrof et al. 2010; Ruthrof et al. 2012), however, adding fertilisers (or surfactants) to the soil surface in a broad scale manner may 1) be expensive, 2) enhance the establishment and growth of invasive species, and 3) not reach the target seedling.

In this study, we investigated the effects of the following treatments on seedling establishment and physiological responses after one year of growth: (a) soil ripping; (b) a fertiliser tablet, placed beneath the seedling at the time of planting; and (c) a surfactant incorporated into a fertiliser tablet. The latter allows transfer of the surfactant from the fertiliser tablet to the seedling's rhizosphere where it can ameliorate hydrophobicity. We hypothesised that ripping would increase rainfall infiltration and hence water availability in these poorly wettable sandy soils. Furthermore, we assumed that surfactant addition would enhance fertiliser efficacy by reducing caking tendencies, thereby facilitating nutrient uptake. Therefore, we anticipated greater survival, more growth as a result of increased photosynthesis (shown in seedling height), better crown health and improved plant water relations in seedlings growing in ripped soil augmented with surfactant-containing fertiliser.

Materials and methods

Study sites

Field trials were established in a closed canopy forest (Ludlow Tuart Forest) and in an open canopy woodland (Yalgorup National Park) to test our hypotheses. The Ludlow Tuart Forest (Ludlow) (2, 049ha) is located 200 km south of Perth, Western Australia, on the southern edge of the Swan Coastal Plain (33°35'S 115°29'E). The nearest weather station with the most complete records is Busselton Shire (009515), which reports an average annual rainfall of 808.7mm, 80% of which falls between May and September (BOM 2012). In the period June 2010 to May 2011, this site received 73% of its annual average rainfall. The soils are classified as the Spearwood Dune System, consisting of variable, though often deep, siliceous, brown and yellow, nutrient

leached sands (McArthur and Bettenay 1974) derived from limestone (Gozzard and Mouritz 1989; McArthur 1991; McArthur and Bettenay 1974). The study site is representative of many of the *E. gomphocephala* woodlands in the region that were logged in the nineteenth and early twentieth century (Heberle 1997) and grazed (predominantly by cattle) since the early 1900s. There are extensive invasions of exotic plants, including arum lily (*Zantedeschia aethiopica*) and black berry nightshade (*Solanum nigrum*). Although Ludlow Tuart Forest has many healthy *E. gomphocephala* adults (canopy cover of $45.9 \pm 2.9\%$, determined with a spherical densitometer at each plot) there is poor natural recruitment and a loss of understorey diversity (DEC 2007).

Yalgorup National Park (Yalgorup) (12, 888 ha) is located 100 km south of Perth, Western Australia, and also lies on the Swan Coastal Plain (115°40'E, 32°45'S). The nearest weather station with the most complete records is Mandurah (009977), which reports an average annual rainfall of 673 mm, which falls in a similar Mediterranean pattern as the Ludlow Tuart forest (BOM 2012 b). In the period June 2010 to May 2011, this site received 71% of its annual average rainfall. The area lies on the Spearwood Dune System (Portlock et al. 1995) and has soils similar to the first study site. The site is typical of many *E. gomphocephala* woodlands in the region, which have been subject to various forms of degradation such as grazing, weed invasion (e.g. dune onion weed, *Trachyandra divaricata*) and changed fire regimes (Archibald et al. 2010). The site was highly degraded with a scattered canopy (canopy cover $4.3 \pm 1.1\%$), a failure of natural recruitment of the canopy species, and a loss of understorey diversity and cover. To avoid kangaroo grazing the trials were fenced. The entirety of both sites were sprayed using 1% Glyphosate™ two weeks prior to planting to control invasive weeds.

Experimental design

At each site, the study was a split-plot design with two soil treatments (ripped and unripped) and three plant treatments: Typhoon (a 20g fertiliser tablet [Sunpalm Australia]); TinkTab (a newly developed 20g fertiliser incorporating a surfactant [Sunpalm Australia]); and, an unfertilised control (no treatment) (see Table 1 for a description of each fertiliser composition). The experimental units were arranged in six blocks (each 12 m x 5 m), each subdivided into three plots (each 4 m x 5 m). The soil treatment, henceforth referred to as 'ripping', was assigned to three blocks, and three blocks were unripped. The three fertiliser treatments were randomly assigned to plots within each block, so that all three fertiliser treatments were replicated three times in the ripped area, and three times in the unripped area. Ripping, using a tractor pulling a prong, took place two weeks prior to planting to a minimum of 30 cm in depth and 1 m spacing between planting rows.

In early June 2010 (early winter), Pottiputki (Lannen Plant Systems, Finland) tree planters were used to plant 20, six-month-old, actively growing, local Swan Coastal Plain provenance, *E. gomphocephala* seedlings grown outside at a local native plant nursery (average ~15cm height) containerized in forestry tubes (5 cm width x 15 cm tall) into each plot at a density of one per square meter to mimic natural recruitment densities (Ruthrof 2003). Seedlings were planted to a depth of 15 cm so that the root ball and top of potting mix was covered by the in situ soil. In the ripping treatment, seedlings were planted at the bottom of the rip-line. Fertiliser tablets were placed beneath the root ball prior to adding the seedling. A total of 720 seedlings (360 seedlings per site) were planted.

Given that the first year is the most critical period for the establishment of planted seedlings (Benayas 1998; Castro et al. 2004b; Savill et al. 1997), monitoring was undertaken in June 2011 (early winter), one year after planting. Above ground measurements were recorded for each plant in each plot and included: survival, height (to the nearest cm) and crown health. Crown health was rated from 1-5; 1 = very poor (including dead individuals), 2 = poor, 3 = fair, 4 = good, and 5 = excellent, taking into account general vigour, colour and amount of herbivory (Ruthrof 1997; Ruthrof et al. 2010). For six live seedlings in each plot (2 small, 2 medium and 2 large), basal stem diameter at 5 cm height above ground level was only measured at the Yalgorup site.

In June 2011 (early winter, when seedlings are at the start of their growing period), stomatal conductance and photosynthesis were measured to understand plant carbon and water relations in response to treatments. Measurements were undertaken over two days per site. Gas exchange was measured using a portable photosynthesis system equipped with the standard leaf chamber fluorometer and an LED light unit (LI-6400XT with 6400-02B LED light source, Licor, Lincoln, Nebraska, USA). Measurements were conducted at ambient air temperature and relative humidity with the leaf chamber CO₂ concentration set to 390 ppm (Ludlow forest: T_{leaf} = 15.5 ± 0.4 °C, air-to-leaf vapour pressure deficit = 0.80 ± 0.02 kPa; Yalgorup woodland: T_{leaf} = 15.3 ± 0.5 °C, air-to-leaf vapour pressure deficit = 0.84 ± 0.03 kPa; mean ± SE). Pre-dawn measurements were performed with the leaf chamber illumination switched off while daytime measurements were taken under saturating light conditions of 1500 μmol m⁻² s⁻¹ provided by the LED light unit (*cf.* Larcher, 2003 provides a compilation of photosynthetic light saturation values across functional groups). At each site, leaf gas-exchange recordings were taken over two consecutive clear sky days with similar temperature and relative humidity. Three out of the six

selected seedlings for each treatment combination were assessed on each day during a measurement period of 90-120 min prior to dawn and between noon and 2 pm, respectively. Given the limited number of leaves, predawn and midday leaf water potential measurements were restricted to one leaf per seedling using a Scholander-type pressure bomb (Model 1000 pressure chamber, PMS instruments, OR, USA) connected to a cylinder filled with industrial grade nitrogen gas. Leaves were removed using a scalpel and then transferred into plastic bags and measured immediately.

The following destructive measurements were only taken at Yalgorup National Park in July 2011 (winter). This time of year was chosen as the deep sandy soils make digging during the summer and autumn months (December-May) difficult due to the risk of holes collapsing. At least two seedlings per plot were carefully excavated using an excavator, shovel and then by hand, and root length noted. Shoots and roots were separated at soil surface. Roots and shoots were bagged, labelled and oven dried for at least three days at 80 °C, after which dry root weights (separated into root length classes: top of root, 0-10 cm and the rest, 10 cm onwards) and shoot weights were recorded.

Soil respiration can change with temperature and soil water availability, mainly due to the activity of soil microbes. The measurements were undertaken in winter, as dry soil conditions could result in very low rates of soil respiration in summer and thus make the measurements less accurate. The measurements were undertaken as outlined in the user manual and the CO₂ concentration at the soil surface was used to select an appropriate setting for the measurement cycles. Soil temperature was measured in close proximity to the soil respiration chamber and

was measured at the same time as soil respiration. Soil rings were put in place 12 hours prior to measurement to prevent interference and were arranged randomly within plots (three per block) in between plants but not directly adjacent to plants (and on rip lines in the ripping treatment).

Data analysis

All statistical computations were performed in R (2.15.3). We applied generalised linear mixed effects models (GLMMs) using Laplace approximation and binomial errors (with logit link) to analyse seedling survival data for each site (R-package *lme4*, (Bates et al. 2013)). The GLMMs contained ‘ripping’ and ‘fertiliser’ and their interaction as explanatory variables. ‘Fertiliser’ (split-plot factor) nested in ‘ripping’ (whole-plot factor) nested in ‘block’ was modelled as random term to reflect the study design. Seedling height, ecophysiological variables and soil respiration were analysed using linear mixed effects models, R-package *nlme*, (Pinheiro et al. 2013) containing the same random error structure. Graphical tools based on plots of the standardised residuals vs. fitted values and against explanatory variables as well as quantile-quantile plots were used for model validation. When heteroscedasticity was detected we used exponential, power, and constant variance functions or a combination thereof to model the heterogeneity patterns, which were mainly due to differing variances among measurement times (pre-dawn vs. midday). Multiple comparisons were performed using Tukey contrasts, R package *multcomp* (Hothorn et al. 2008) and the resulting P-values were adjusted using the Benjamini and Yekutieli method (Benjamini 2001).

Crown health was assessed on a 5-point ordinal scale and therefore analysed using cumulative link mixed models, CLMM, R-package *ordinal* (Christensen 2013). The CLMMs were run with a

logit link, flexible thresholds and the same fixed and random terms as described for the previous mixed-effects models. Since there is no multiple comparison procedure available for CLMMs, we created the required contrasts ‘by hand’ by alternately merging two fertiliser treatments. We then ran a series of models with merged fertiliser treatments and applied likelihood ratio tests to compare these restricted models with the original model that allowed individual parameters for all three fertiliser treatments (control, TinkTab, Typhoon). The resulting P-values were adjusted for multiple comparisons using the Benjamini and Yekutieli method (Benjamini 2001). For all models, the significance of the fixed effects was assessed using a backwards selection procedure based on likelihood ratio tests (Zuur et al. 2009).

Results

Survival

At Ludlow forest, 61% of all seedlings were alive, one year after planting (Fig. 1). Seedling survival remained unaffected by fertiliser application but significantly more seedlings survived when the soil was ripped prior to planting (on average 83% survival in ripped vs. 38% survival in unripped soil, Table 2). At the Yalgorup woodland site, 69% of the seedlings were alive after one year and there was a trend towards greater survival when fertiliser was applied (Table 2).

Although more seedlings survived on ripped soil, especially in combination with fertiliser application, ripping had no statistically significant impact on seedling survival. Ripping also reduced the variation in responses to treatments.

Seedling height

At Ludlow forest, both soil ripping and fertiliser application significantly stimulated seedling height (Table 2, Fig. 2). In the control treatment, seedlings in ripped soil grew about twice as tall compared to those in unripped soil. Fertilised seedlings in ripped soil grew 2.35 times the height of those in unripped soil, regardless of fertiliser type. Both fertilisers enhanced height significantly compared to control seedlings (+39-49%) but there was no significant difference in plant height between Typhoon and TinkTab.

By contrast, at Yalgorup, soil ripping did not stimulate seedling height, while fertiliser application resulted in significantly taller seedlings (Table 2, Fig. 2). Seedlings treated with TinkTab fertiliser grew on average 37% taller and those treated with Typhoon 54% taller than control seedlings. However, these differences between fertilisers were statistically not significant.

Seedling basal diameter

In Yalgorup, stem diameter at 5 cm height increased significantly with fertiliser application reaching 1.6-1.7 times the values seen in the control seedlings (Control $3.78\text{cm} \pm 0.41^{\text{a}}$, TinkTab $6.08 \pm 0.98^{\text{b}}$, Typhoon $6.54 \pm 0.81^{\text{b}}$, different lower case letter indicates statistically significant differences at $\alpha = 0.05$, multiple comparison test with Tukey contrasts). There was neither a significant fertiliser \times ripping interaction nor a significant ripping effect detectable (Table 2).

Crown health

At Ludlow forest, soil ripping had a very strong effect on seedling crown health (Table 2, Fig. 3a). While the vast majority of seedlings growing in unripped soil showed poor crown health, the

crowns of seedlings growing in ripped soil were predominantly healthy (scored fair or better). Fertiliser application also had a positive effect on crown health (Table 2) but there was no indication of a ripping × fertiliser interaction (Table 2). The rather high proportion of seedlings with very poor crown health in unfertilised, unripped soil (c. 80%) was significantly reduced by fertiliser application. The strongest effect was seen with Typhoon fertiliser, which reduced the proportion of the lowest crown health score by nearly 50% compared to unfertilised control seedlings. In ripped soil, only 12 to 22% of the seedlings showed very poor crown health. Nevertheless, here fertiliser application also improved crown health. The proportion of seedlings assigned the best crown health score increased from c. 17% in unfertilised seedlings to around 50% in the fertilised treatment groups. A similar pattern, albeit less pronounced, was observable in the second highest crown health score. Overall, the two fertiliser types did not differ significantly in their beneficial effect on crown health (Table 2).

At Yalgorup, both ripping and fertiliser application significantly improved crown health (Table 2) but there was no evidence of an interaction between the two treatments (Table 2, Fig. 3b). Ripping greatly reduced the proportion of seedlings in the lowest crown health score and increased the fraction of healthier seedlings, particularly the ones showing 'good' crown health. In unripped soil, fertiliser application reduced the proportion of seedlings exhibiting very poor crown health from around 60% by roughly a third, regardless of fertiliser type. However, Typhoon fertiliser produced the largest number of seedlings showing excellent crown health (38%). In ripped soil, fertilisation decreased the proportion of seedlings in the lowest crown health score by more than half while increasing the proportion of seedlings showing excellent crown health from less than 5% to about 40%, irrespective of fertiliser type. Overall, the

fertiliser-induced crown health improvement did not vary significantly with fertiliser type (Table 2).

Above- and below ground biomass

Logistic constraints confined the biomass harvesting campaign to the Yalgorup site. Shoot and root biomass remained unaffected by ripping but more than doubled when fertiliser was applied, irrespective of fertiliser type (Table 2, Fig. 4a). Roots in the upper 10 cm of the soil profile made up 50-55% of the total root mass, except for the unripped plots fertilised with Typhoon where roots in the upper soil horizon only contributed 38% to the total root mass (small arrowheads in Fig. 4a separate root mass above and below 10 cm depth). Root:shoot ratios varied between 0.38 to 0.57 but did not show a significant response to ripping or fertiliser application (Fig. 4b).

Typically, single (or occasionally double tap) roots extended well into the soil profile. The longest root measured was 316 cm (ripped soil with Typhoon tablet treatments). Fertiliser application also seemed to stimulate root length but due to the large within-group variation (Control $89.85\text{cm} \pm 6.36^{\text{a}}$, TinkTab $120.85\text{cm} \pm 10.03^{\text{ab}}$, Typhoon $143.55\text{cm} \pm 42.49^{\text{b}}$), differences between the controls and the fertilised seedlings remained only marginally significant or non-significant. There was neither a significant fertiliser \times ripping interaction nor a significant soil ripping effect detectable (Table 2).

Leaf water potential

At Ludlow forest, leaf water potential (Ψ_{leaf}) remained unaffected by fertiliser application but seedlings growing in unripped soil had significantly lower (more negative) pre-dawn and midday

Ψ_{leaf} than seedlings growing in ripped soil (Fig. 5a). These differences in Ψ_{leaf} grew larger over the day resulting in a significant ripping \times time of day interaction (Table 2). Midday Ψ_{leaf} of seedlings on ripped soil dropped only to about -1 MPa whereas seedlings in unripped soil showed Ψ_{leaf} between -1.5 and -1.75 MPa.

At Yalgorup woodland, Ψ_{leaf} was similar across fertiliser and ripping treatments and only time of day had a significant effect (Table 2). At pre-dawn, Ψ_{leaf} was close to zero and declined to values between -1.4 and -2.0 MPa at midday (Fig. 5a). Hydraulic conductivity of *E. gomphocephala* seedlings assessed in the same study area ranged from 0.5 to 0.65 kg m⁻¹ MPa⁻¹ s⁻¹ per unit stem area (K_s) and from 7.5×10^{-5} to 1.0×10^{-4} kg m⁻¹ MPa⁻¹ s⁻¹ per unit leaf area (K_L) depending on the depth of the groundwater table (Drake et al. 2011).

Stomatal conductance and photosynthesis

At Ludlow forest, fertiliser application did not have a significant impact on stomatal conductance but there was a significant overall ripping (Table 2) as well as a time of day effect. Seedlings growing on ripped soil had on average 56% and 52% higher stomatal conductance than seedlings in unripped soil at pre-dawn and midday, respectively (Fig. 5b). Midday stomatal conductance was 47% higher compared to pre-dawn regardless of soil ripping. Leaf respiration at pre-dawn varied from 1.1 to 1.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and midday photosynthetic rates from 6.4 to 11.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Control seedlings and those receiving Typhoon fertiliser showed very similar respiration and photosynthesis rates but seedlings treated with TinkTab fertiliser had c. 70% higher photosynthesis rates on ripped compared to unripped soil (Fig. 5c). However, due to the large variation seen in seedlings on unripped soil, these differences were statistically not significant.

At Yalgorup woodland, stomatal conductance at pre-dawn could not be reliably measured due to heavy dew condensation. At midday, rates of stomatal conductance varied between 160 and 200 $\text{mmol m}^{-2} \text{s}^{-1}$ but did not differ significantly across treatments (Table 2, Fig. 5b). Similarly, respiration and photosynthesis rates did not differ significantly across fertiliser and ripping treatments (Fig. 5c). Respiration rates at pre-dawn reached between 1 and 1.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and midday photosynthesis rates varied between 12 and 16 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Soil respiration

Forest soil CO_2 efflux at the Ludlow site averaged 2.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (equivalent to 11 $\text{g C h}^{-1} \text{ha}^{-1}$) during midday and remained unaffected by ripping and fertilisation (Fig. 6). By comparison, soil respiration rates in the woodland at Yalgorup were only about half as high but there ripping stimulated soil respiration significantly by 12%, driven by slightly higher rates in the control plots and those treated with Typhoon fertiliser (Fig. 6). Similar to the forest site, fertiliser application had no significant effect on soil CO_2 release.

Discussion

High seedling mortality due to drought is a key factor in limiting restoration success in MCEs around the world (Castro et al. 2004a). Promoting seedling establishment and enhancing seedling vigour is therefore essential to MCE restoration projects, especially considering that resistance and resilience to extreme events will become increasingly important under future climate scenarios. In this study we show that restoration of degraded *Eucalyptus* ecosystems can be greatly enhanced through site preparation techniques and the addition of nutrient sources.

Although there were site-specific responses [see discussion on site context in Standish et al. (2012) and Ruthrof et al. (2013a)], the key patterns of early survival, height and crown health as well as leaf water potential, stomatal conductance and soil respiration (at Yalgorup) were enhanced through the ripping of deep sandy soils. The addition of either fertiliser enhanced seedling growth, crown health and shoot growth and root biomass. The newly developed fertiliser, TinkTab, increased vigour to a similar level as the Typhoon tablet, yet contains fewer nutrients, suggesting that improved moisture in the profile, together with mobilisation of limited nutrients could facilitate seedling establishment; however, this requires further testing.

It is widely accepted that early seedling survival and vigour can be achieved with deep ripping in a restoration context (Bocio et al. 2004; Espelta et al. 2003; Li et al. 2003; Lof et al. 2012). For example, deep ripping has produced positive results in restoration of *Pinus nigra* forest in NE Spain and in *Eucalyptus blakelyi* in eastern Australia (Espelta et al. 2003; Li et al. 2003). In this study, the site preparation technique of ripping had a positive effect on early seedling establishment in terms of survival, height and crown health at Ludlow and crown health at Yalgorup. Perhaps the differences in extant canopy cover and hence competing vegetation could explain some difference between the sites. In addition, the variation is much smaller in ripped plots compared with unripped plots (presumably ripping reduces heterogeneity within the soil profile). Previous work has shown that neither site is compacted; yet this technique alters moisture availability within the soil profile, promoting deeper root length and increasing revegetation success (Ruthrof et al. 2013a). Root biomass did not differ significantly between ripping and control treatments; thus, root size and length is unlikely to explain greater seedling survival and vigour rates. Therefore, we can assume improved soil physical properties as a result

of ripping, such as enhanced infiltration of rainfall, and thus greater water and nutrient availability further into the soil profile, especially in sites with higher levels of competition from surrounding canopy. Increases in soil moisture commonly translate into higher nutrient availability, since nutrients are only available for plant uptake in soil solution.

Physiological responses to site preparation and treatments also clearly demonstrated that ripping had a strong positive effect on seedling water relations at the Ludlow site. Favourable leaf water status and higher stomatal conductance in ripped plots suggest that this treatment improved soil hydrological properties; presumably by facilitating rainfall infiltration and thus alleviating soil moisture constraints. Higher interception and greater below ground competition for water at Ludlow (which has a much higher canopy cover) seem plausible causes for the pronounced seedling response to ripping. Other studies have noted enhanced responses in water relations to improved environmental conditions resulting from site preparation; Fleming et al. (1996) for example, found that ripping, scalping or herbicide treatments consistently enhanced stomatal conductance and transpiration in the first growing season in *Pseudotsuga menziesii* var. *glauca* and *Pinus contorta* var. *latifolia* seedlings compared with seedlings in control plots. In contrast, the hydraulic conditions at the open woodland setting at Yalgorup (with a lower canopy cover) seem to be more homogeneous, that is, it has less interception and less root competition (i.e. competition for water) from large neighbouring trees. Therefore, ripping may not produce a noticeable effect on water relations there and thus, the lower bulk soil density and little prior impediment to infiltration render ripping unnecessary.

The addition of nutrients is known to enhance the growth of particular species in a restoration context; in degraded eucalypt woodlands in Western Australia (Ruthrof et al. 2010; Ruthrof et al. 2013a), in reforestation of *Pinus halepensis* and *Acacia salicina* in southeastern Spain (Oliet et al. 2005; Oliet et al. 2009), and under post mining conditions in Portugal (Clemente et al. 2004) and Western Australia (Koch and Samsa 2007; Ruthrof 1997). In the current study, the addition of nutrients (in either form) clearly enhanced the height, crown health, shoot and root biomass and root length of seedlings. Low levels of health were visually and clearly associated with nutrient deficiencies (distinct colouration of the leaves) and drought (desiccation of leaves). However, increases in soil nutrient supply may also have adverse effects such as the invasion of nutrphilous species reported from the South African fynbos (Yelenik et al. 2004).

We suspected nutrient-limitation of photosynthesis on the nutrient-impooverished soils of the coastal plain (Grigg et al. 2009; Lambers et al. 2010) and thus anticipated fertiliser-driven increases in photosynthetic capacity. However, at neither site did light-saturated rates of photosynthesis respond to fertiliser application. The average photosynthetic rates at Ludlow forest and Yalgorup woodland (10 and $14 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) were in the range of maximal rates reported for broadleaved trees (Larcher 2003), suggesting no nutrient-limitation of photosynthesis, which, given the nutrient-poor soils, implies high nutrient uptake efficiency, which has been shown for many Western Australian species (Lambers et al. 2010).

Hydrophobic soils can decrease seedling survival by decreasing soil moisture availability, and thus could act as an ecological threshold by impeding establishment (Madsen et al. 2012b). However, surfactants can enhance infiltration and improve water availability for seedling

survival and plant growth (Madsen et al. 2012a; Madsen et al. 2012b). The present study tested whether the surfactant, newly developed to be incorporated into a standard fertiliser tablet and placed beneath the seedling at the time of planting, could increase seedling survival and vigour. Results showed that the new fertiliser incorporating a surfactant significantly increased the vigour of seedlings compared with the control, and similar to the responses to the original fertiliser it was based upon, yet contains fewer nutrients, however, this requires further investigation.

Site treatments may have significant effects on soil respiratory processes; fertiliser or organic matter application, for example, has been shown to increase microbial activity and hence soil respiration (Fereidooni et al. 2013; Treseder and Allen 2000). However, and somewhat surprisingly, higher seedling survival and increased height on ripped and fertilised soil in the Ludlow forest did not translate into higher rates of soil respiration. This suggests that ripping-induced aeration effects in this type of soil may be short-lived and confirms the results of previous studies where nutrient addition failed to stimulate soil respiration (Allison et al. 2010). However, it must be noted that soil respiration is extremely variable at a seasonal scale and thus further work should focus on seasonal variations between site treatments. Furthermore, given that the fertiliser tablet was placed beneath each seedling, the fertilisation effect may have been highly localised and not facilitated a general soil response.

Given the large increase in above- and below ground biomass associated with fertiliser application at Yalgorup, one would expect increases in soil CO₂ efflux due to greater root respiration, but we observed no fertiliser-driven stimulation in soil respiration. A simultaneous

decrease in microbial respiration in response to nutrient addition could have compensated likely increases in respiratory CO₂ release from roots and thus explain the apparent lack of response. Though, taking into consideration the root architecture we encountered (very thin and long single or double tap roots) it is not surprising that we did not capture a larger signal from soil close to seedlings growing in ripped soil.

This study has shown that in deep sandy soils, commonly thought to have high levels of penetrability and aeration, site preparation in the form of ripping, and nutrient addition increased above and below ground vigour of planted seedlings in a restoration context. Targeting the co-limitation by water and nutrients greatly increased the chances of successful restoration in these Mediterranean climate ecosystems. The site and plant treatments outlined in this study, as well as the use of physiological measurements to determine seedling and site responses, have great potential to help increase restoration success in these types of ecosystems.

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Compliance with Ethical Standards

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Table 1. Description of fertiliser treatments and constituents applied in restoration trials using *Eucalyptus gomphocephala* seedlings within Ludlow Forest and Yalgorup Woodland, Western Australia

No.	Treatment	Constituents
1	Control	No treatment applied
2	Fertiliser tablet Typhoon + Surfactant (20g) TinkTab. Sunpalm Australia, Wangara, WA	Total N 10.1%, Total P 2.0%, Total K 4.16%, S 1.78%, Mg 0.38%, Fe 0.195%, Zn 0.04%, Cu 0.04%, Mn 0.04%, B 0.005%, Mo 0.003%, non-ionic surfactant.
3	Fertiliser tablet Typhoon (20g): Sunpalm Australia, Wangara, WA	Total N 20%, Total P 4.4%, K 8%, S 4.12% and Mg 0.36%, Cu 0.17%, Zn 0.32%, Fe 0.36%, Mn 0.17%, Mo 0.01% and B 0.02%.

Table 2. Results of backwards selection procedures based on likelihood ratio tests, applied to linear and generalised linear mixed-effects models. *L* = likelihood ratio statistic, *DF* = degrees of freedom of the likelihood ratio statistic, *P* = P-value, *** *P* < 0.001, ** *P* < 0.01, * *P* < 0.05; α = 0.05. Note that main effects that are part of a significant interaction term are not interpretable on their own and in these cases are hence no P-values for main effects given (Zurr et al. 2009). The collection of above- and below-ground biomass and root related data was restricted to the Yalgorup woodland site.

Dropped term	Ludlow forest			Yalgorup woodland		
	<i>L</i>	<i>DF</i>	<i>P</i>	<i>L</i>	<i>DF</i>	<i>P</i>
Survival						
Ripping x fertiliser	1.73	2	0.421	1.60	2	0.450
Ripping	5.18	1	0.023 *	1.87	1	0.172
Fertiliser	0.57	2	0.754	4.64	2	0.098
Seedling height						
Ripping x fertiliser	4.41	2	0.110	4.76	2	0.092
Ripping	12.97	1	< 0.001 ***	0.01	1	0.908
Fertiliser	10.39	2	0.006 **	35.76	2	< 0.001 ***
Crown health						
Ripping x fertiliser	3.08	2	0.215	1.48	2	0.478
Ripping	8.92	1	0.003 **	5.25	1	0.022 *
Fertiliser	11.25	2	0.004 **	16.08	2	< 0.001 ***
Seedling basal diameter						
Ripping x fertiliser	–	–	–	0.67	2	0.716
Ripping	–	–	–	0.05	1	0.825
Fertiliser	–	–	–	18.79	2	< 0.001 ***
Shoot biomass						
Ripping x fertiliser	–	–	–	2.45	2	0.293
Ripping	–	–	–	2.88	1	0.090
Fertiliser	–	–	–	62.48	2	< 0.001 ***
Root biomass (top 10 cm)						
Ripping x fertiliser	–	–	–	3.74	2	0.154
Ripping	–	–	–	0.09	1	0.761
Fertiliser	–	–	–	43.33	2	< 0.001 ***
Root biomass (below 10 cm)						
Ripping x fertiliser	–	–	–	5.47	2	0.065
Ripping	–	–	–	0.03	1	0.855

Fertiliser	–	–	–	37.60	2	< 0.001	***
Total root biomass							
Ripping x fertiliser	–	–	–	2.09	2	0.352	
Ripping	–	–	–	0.76	1	0.383	
Fertiliser	–	–	–	61.95	2	< 0.001	***
Root : shoot							
Ripping x fertiliser	–	–	–	1.14	2	0.565	
Ripping	–	–	–	0.60	1	0.439	
Fertiliser	–	–	–	4.18	2	0.124	
Root length							
Ripping x fertiliser	–	–	–	0.65	2	0.723	
Ripping	–	–	–	0.25	1	0.615	
Fertiliser	–	–	–	5.50	2	0.064	
Leaf water potential (Ψ_{leaf})							
Ripping x fertiliser x daytime	2.72	2	0.256	5.39	2	0.067	
Fertiliser x daytime	0.29	2	0.863	9.27	2	0.205	
Ripping x daytime	35.35	1	< 0.001 ***	0.44	1	0.508	
Ripping x fertiliser	3.97	2	0.137	1.53	2	0.465	
Fertiliser	1.13	2	0.570	2.78	2	0.249	
Ripping	–	–	–	3.20	1	0.074	
Daytime	–	–	–	121.59	1	< 0.001	***
Stomatal conductance (g_s)							
Ripping x fertiliser x daytime	2.58	2	0.275	Pre-dawn dew formation, hence no factor 'daytime'			
Fertiliser x daytime	2.73	2	0.256				
Ripping x daytime	0.02	1	0.883				
Ripping x fertiliser	0.03	2	0.984	1.33	2	0.515	
Fertiliser	2.19	2	0.334	0.45	2	0.799	
Ripping	4.72	1	0.030 *	0.03	1	0.869	
Daytime	16.83	1	< 0.001 ***				
Light-saturated photosynthesis (A_s)							
Ripping x fertiliser x daytime	2.63	2	0.269	0.06	2	0.971	
Fertiliser x daytime	2.98	2	0.226	3.27	2	0.195	
Ripping x daytime	1.38	1	0.241	1.41	1	0.234	
Ripping x fertiliser	1.38	2	0.501	0.35	2	0.839	
Fertiliser	0.47	2	0.792	2.20	2	0.332	
Ripping	0.18	1	0.668	1.95	1	0.163	
Daytime	248.72	1	< 0.001 ***	247.30	1	< 0.001	***
Soil respiration							
Ripping x fertiliser	0.19	2	0.910	2.50	2	0.287	
Ripping	0.04	1	0.849	5.77	1	0.016	*
Fertiliser	2.32	2	0.314	2.81	2	0.246	

Figure Legends

Fig. 1 Survival (%) of *Eucalyptus gomphocephala* seedlings grown under different soil and fertiliser treatments after one year at Ludlow Forest (closed canopy) and Yalgorup Woodland (open canopy), Western Australia. Means \pm SE, $n = 3$ blocks. * $P < 0.05$, $\alpha = 0.05$.

Fig. 2 Height (cm) of *Eucalyptus gomphocephala* seedlings grown under different soil and fertiliser treatments after one year at Ludlow Forest (closed canopy) and Yalgorup Woodland (open canopy), Western Australia. Means \pm SE, $n = 3$ blocks. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $\alpha = 0.05$. Significant fertiliser effects were followed up with a multiple comparison procedure using Tukey contrasts.

Fig. 3 Crown health of *Eucalyptus gomphocephala* seedlings grown under different soil and fertiliser treatments after one year at Ludlow Forest (closed canopy) (a) and Yalgorup Woodland (open canopy) (b), Western Australia. Means, $n = 3$ blocks, $\alpha = 0.05$.

Fig. 4 Shoot and root dry mass (g) (a) and root: shoot ratio (b) of *Eucalyptus gomphocephala* seedlings grown under different soil and fertiliser treatments after one year at Yalgorup Woodland (open canopy), Western Australia. The small arrowheads in (a) mark the boundary between root dry mass derived from above or below 10 cm soil depth. Means \pm SE, $n = 3$ blocks. Different lower case letters indicate statistically significant differences at $\alpha = 0.05$ (multiple comparison procedure using Tukey contrasts).

Fig. 5 Pre-dawn and midday leaf water potential, Ψ_{leaf} (a), stomatal conductance, g_s (b) and light-saturated photosynthesis, A_s (c) of *Eucalyptus gomphocephala* seedlings grown under different soil and fertiliser treatments after one year at Ludlow Forest (closed canopy) and Yalgorup Woodland (open canopy), Western Australia. Means \pm SE, $n = 3$ blocks. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different lower case letters indicate statistically significant differences at

$\alpha = 0.05$ (multiple comparison procedure using Tukey contrasts). The dotted line in panel a) indicates 50% loss of hydraulic conductivity (Franks et al. 2007).

Fig. 6 Soil respiration in plots with *Eucalyptus gomphocephala* seedlings grown under different soil and fertiliser treatments after one year at Ludlow Forest (closed canopy) and Yalgorup Woodland (open canopy), Western Australia. Means \pm SE, $n = 3$ blocks. * $P < 0.05$.

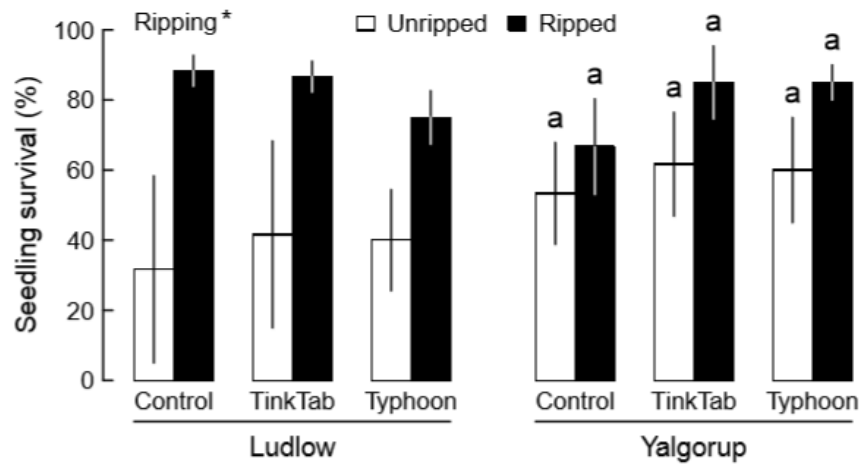


Figure 1.

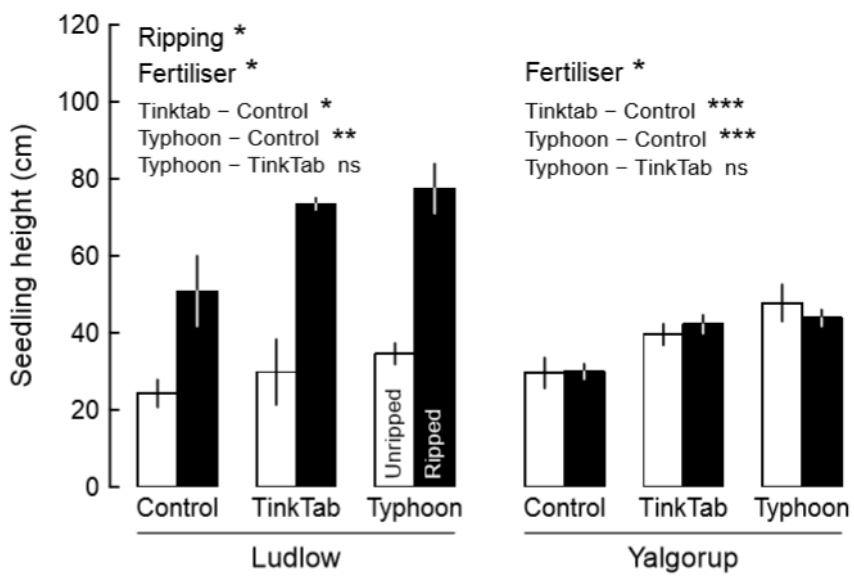


Figure 2.

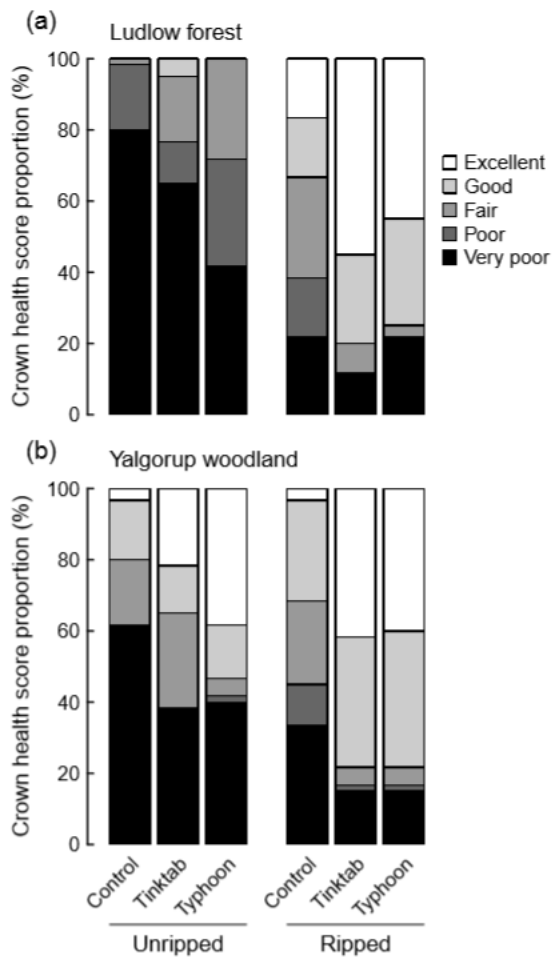


Figure 3.

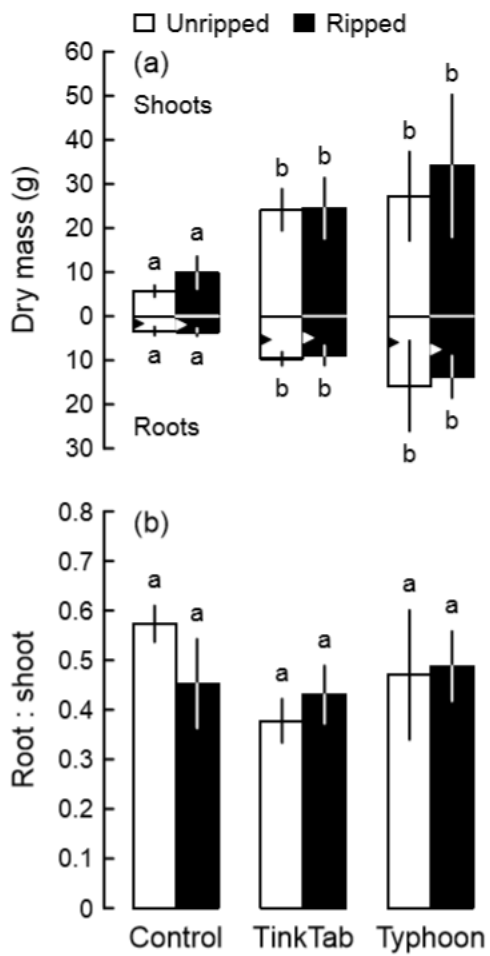


Figure 4.

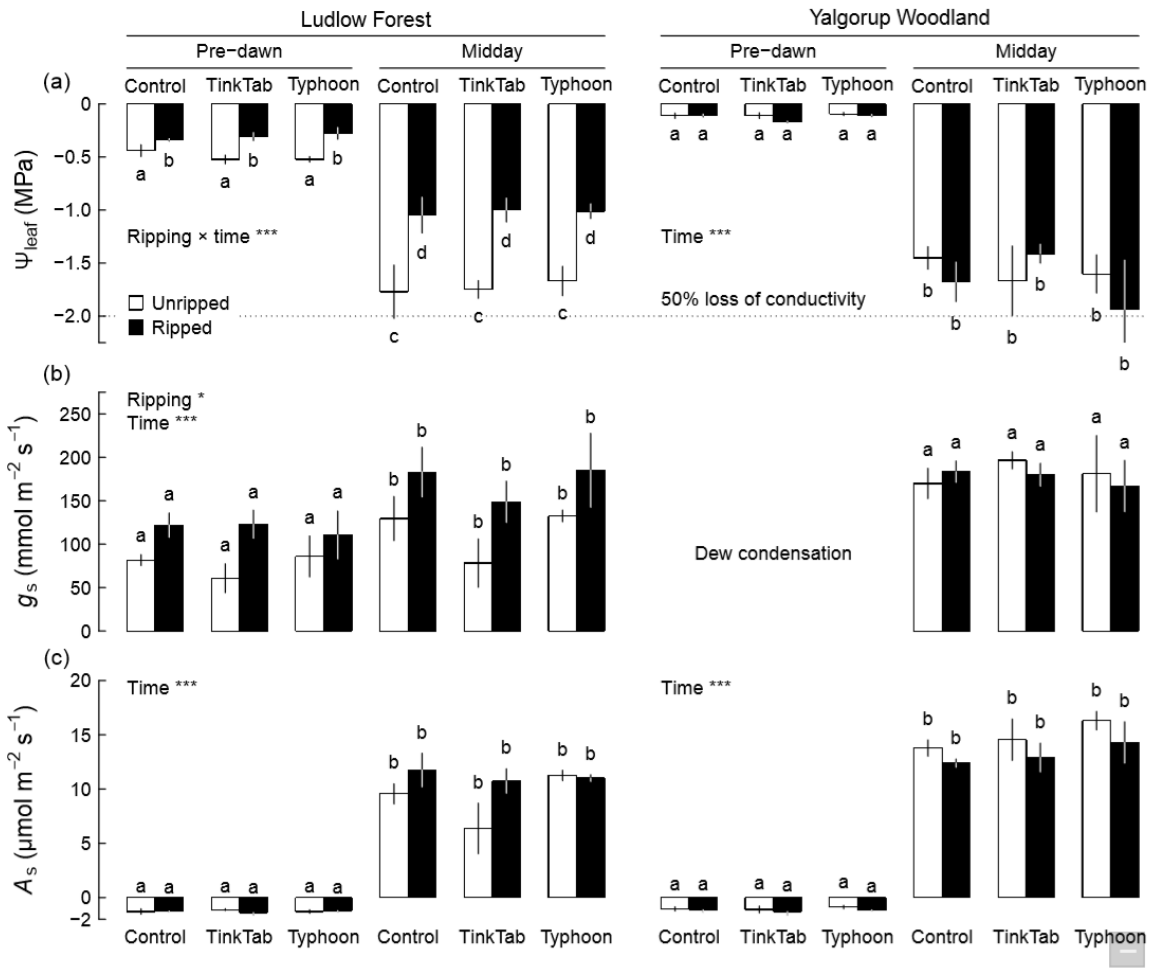


Figure 5.

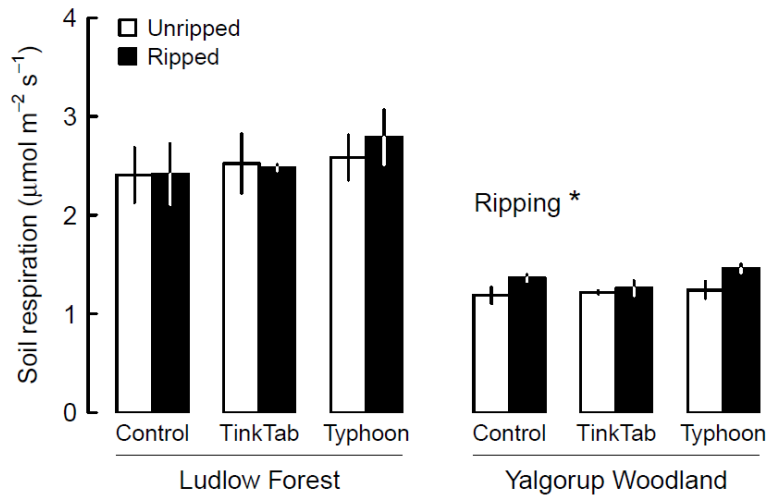


Figure 6.