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Research paper

Transpiration and water-use efficiency in mixed-species forests versus monocultures: effects of tree size, stand density and season

David I. Forrester^{1,2}

¹Chair of Silviculture, Faculty of Environment and Natural Resources, Freiburg University, Tennenbacherstr. 4, 79108 Freiburg, Germany; ²Corresponding author (david.forrester@waldbau.uni-freiburg.de)

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Mixtures can be more productive than monocultures and may therefore use more water, which may make them more susceptible to droughts. The species interactions that influence growth, transpiration and water-use efficiency (WUE, tree growth per unit transpiration) within a given mixture vary with intra- and inter-annual climatic variability, stand density and tree size, but these effects remain poorly quantified. These relationships were examined in mixtures and monocultures of *Eucalyptus globulus* Labill. and *Acacia mearnsii* de Wildeman. Growth and transpiration were measured between ages 14 and 15 years. All *E. globulus* trees in mixture that were growing faster than similar sized trees in monocultures had higher WUE, while trees with similar growth rates had similar WUE. By the age of 14 years *A. mearnsii* trees were beginning to senesce and there were no longer any relationships between tree size and growth or WUE. The relationship between transpiration and tree size did not differ between treatments for either species, so stand-level increases in transpiration simply reflected the larger mean tree size in mixtures. Increasing neighbourhood basal area increased the complementarity effect on *E. globulus* growth and transpiration. The complementarity effect also varied throughout the year, but this was not related to the climatic seasonality. This study shows that stand-level responses can be the net effect of a much wider range of individual tree-level responses, but at both levels, if growth has not increased for a given species, it appears unlikely that there will be differences in transpiration or WUE for that species. Growth data may provide a useful initial indication of whether mixtures have higher transpiration or WUE, and which species and tree sizes contribute to this effect.

Keywords: biodiversity, complementarity, facilitation, plant–climate interactions, plant–plant interactions, production ecology.

Introduction

An argument often used in favour of mixed-species forests is their greater productivity. However, faster growing trees generally use more water than slower growing trees (Law et al. 2002), which may in turn make mixtures more susceptible to drought than monocultures and reduce water availability for other users. Despite this potential problem, few studies have compared the transpiration (E_T), water-use efficiency (WUE, tree or stand growth per unit E_T) and their seasonality in mixtures and monocultures. Table 1 summarizes these studies. Most focused on the species stand-level or total stand-level, and provided little information about tree-level patterns, such as

whether relationships between individual tree size and E_T or WUE vary between mixtures and monocultures. However, there is considerable spatial and temporal variability in soil and canopy conditions within mixed-species forests (Canham et al. 1999, Schume et al. 2004, Boyden et al. 2012) and this is likely to be reflected in tree-level relationships. Given that stand-level patterns are determined by the growth patterns of the individual trees and the interactions between them, the tree-level patterns include fundamental information about the processes underlying the species-level and total stand-level responses. They also provide valuable information for making management decisions. Tree-level patterns of water-related

Table 1. Experiments that have compared the transpiration or drought stress of mixed-species with monospecific forests. The experiment in Study 2 was used for this study. *E.g.* *Eucalyptus globulus* Labill.; *A.m.* *Acacia mearnsii* de Wildeman; *P.m.* *Pseudotsuga menziesii* (Mirb.) Franco; *A.r.* *Alnus rubra* Bong.; *Pa.* *Picea abies* (L.) Karst. *F.s.* *Fagus sylvatica* L.; *Q.c.* *Quercus cerris* L.; *Q.p.* *Quercus petraea* Liebl.; *B.p.* *Betula pendula* Roth; *SFD*, sap flux density.

Study	Allowed stand density to vary naturally?	Species	Climatic zone	Stand characteristics	Experimental design	Results	References
1	Yes	<i>Anacardium excelsum</i> (Bertero & Balb. ex Kunth) Skeels, <i>Cedrela odorata</i> Liné, <i>Hura crepitans</i> Linné, <i>Luehea seemannii</i> Triana & Planch, <i>Tabebuia rosea</i> (Bertol.) DC	Tropical (Panama). Mean annual precipitation of 2300 mm	Plantation. 7 years old. 3-m spacing (1111 trees ha ⁻¹)	Monocultures of five species, 3 two-species mixtures, 3 three-species mixtures, and 1 five-species mixture. <i>n</i> = 1 for each species combination except the five-species mixture where <i>n</i> = 4. For each diversity level <i>n</i> = 3 or 5. Measured 4 trees per species per treatment, and measurements were done for 1 year	Compared with monocultures, annual E_T in 2- and 3-species mixtures was 1.4% higher and in five-species was 56% higher. WUE was 2.1, 3.7, 5.7, 3.4 g DM per kg water in 1-, 2-, 3- and 5-species mixtures, respectively. There were inter-specific differences in the depth of water uptake, but inter-specific interactions did not influence these differences. The lower WUE in the five-species mixture was suggested to result from a reduction in light-use efficiency or from nutrient limitation. Higher E_T in the 5-species mixture was suggested to result from complementary water use or enhanced energy exchange through higher canopy roughness, although this proposed canopy roughness effect was not calculated and no significant difference was found in mixtures of Study 2 that also had distinctly stratified canopies	Kunert et al. (2012), Schwendenmann et al. (2015)
2	Yes	<i>Eucalyptus globulus</i> , <i>Acacia mearnsii</i>	Temperate (Australia). Mean annual precipitation of 1009 mm but during the 1-year study period it was 730 mm	Plantation. 14 years old. Spacing 3.3 × 2 m (1515 trees ha ⁻¹)	Monocultures and 1 : 1 mixtures (<i>n</i> = 3 for each plot-level treatment). 16–18 trees per species per treatment were measured over the course of a year (different trees measured at different times of the year)	Above-ground biomass growth (Mg ha ⁻¹) was higher in mixtures (<i>E.g.</i> 4.8 + <i>A.m.</i> 0.9) than <i>E.g.</i> (3.3) or <i>A.m.</i> monocultures (1.6). Annual E_T (mm) was higher in mixtures (<i>E.g.</i> 285 + <i>A.m.</i> 134) than <i>E.g.</i> (358) and <i>A.m.</i> (217) monocultures. WUE was higher in mixtures than monocultures because the WUE of <i>E.g.</i> in mixtures (1.69 kg above-ground biomass per cubic metre water transpired) was higher than in monocultures (0.94). Growth, E_T and WUE increased due to increased N and P availability, and light absorption, which increased photosynthesis of <i>E.g.</i> and shifted biomass allocation from below to above-ground. Soil moisture profiles indicated that there was also reduced competition for water from <i>A.m.</i>	Forrester et al. (2010) (water-related interactions). Also, see Forrester et al. (2004, 2005, 2006, 2007, 2011, 2012c)
3	Yes	<i>Pseudotsuga menziesii</i> , <i>Alnus rubra</i>	Temperate (USA). Mean annual precipitation of 2300 mm	Plantation. 15 years old. 3 × 3 m spacing (1111 trees ha ⁻¹)	Monocultures of each species, and even-aged mixtures where <i>A.r.</i> was 5 years younger. This was replicated on two sites, differing in productivity (eight plots in total, <i>n</i> = 1 for each treatment). Measured eight trees per plot (four per species in mixtures) for 1 year	19% of <i>P.m.</i> E_T occurred while <i>A.r.</i> was leafless, and the peak in E_T for <i>P.m.</i> was in early July while that of <i>A.r.</i> was in late May. At the poorer site monocultures had higher basal area, leaf areas, and E_T , but the statistical significance could not be tested. At the other site the <i>A.r.</i> had the highest basal area (not LA) and E_T , and the even-aged mixed stand was the second highest. E_T was correlated with above-ground biomass and to a lesser extent with growth. The poorer site had a lower WUE but the relationship between biomass and E_T was the same for all species and treatments. There was no evidence of N fixation by <i>A.r.</i>	Moore et al. (2011)

4	No, similar leaf area index (7.1–7.9)	<i>Picea abies</i> , <i>Fagus sylvatica</i>	Temperate (Austria). Mean annual precipitation of 850 mm	Forest. About 60 years old	One mixture and a monoculture of each species ($n = 1$ for each treatment). For a wet year and a dry year high temporal and spatial resolution soil moisture data was used to examine soil moisture dynamics. Interception was also calculated	Soil moisture depletion data indicated that <i>F.s</i> had a high E_T per area. Depletion and recharge of mixtures were not additive effects of monocultures. Water storage under the mixtures was similar to that of <i>F.s</i> monocultures, and during several dry periods the extraction in these plots was about 45% higher than <i>Pa</i> monocultures. The mixtures extracted a higher proportion of water from deep soil layers than the monocultures. The greater than expected evapotranspiration in mixtures was mainly due to the <i>F.s</i> , which deepened and intensified its root system in the mixtures, while that of <i>Pa</i> became shallower. The <i>Pa</i> monocultures, which had the lowest LAI, had higher interception rates even when <i>F.s</i> had leaves. Soil moisture content was often lowest under <i>Pa</i> and highest in <i>F.s</i> monocultures, except during the driest times, when there were no differences. Therefore <i>Pa</i> was stressed earlier in the mixtures. The higher interception by <i>Pa</i> was largely compensated for by the higher E_T and competitive ability of <i>F.s</i>	Schume et al. (2004)
5	No, similar basal areas (36–45 m ² ha ⁻¹)	<i>Fagus sylvatica</i> , <i>Tilia</i> sp., <i>Fraxinus excelsior</i> L., <i>Carpinus betulus</i> L., <i>Acer</i> sp.	Temperate (Germany). Mean annual precipitation of 590 mm. During the drier year of measurement 518 mm	Forest. 83–166 years	A total of three plots, including a <i>F.s</i> monoculture, a mixture containing three species (70% basal area was <i>F.s</i>) and another with five species (<10% basal area was <i>F.s</i>) ($n = 1$). Measured 3–8 trees per species per treatment during an average growing season and again during the following drier growing season	During the summer with average rainfall, E_T was 50% higher in the 5-species mixture than in the plots with higher proportions of <i>F.s</i> . During the dry summer E_T of all plots was similar. The E_T of the 3-species plot was 43% higher during the dry year than it was in the average year. As in each of the other studies, there were large inter-specific differences in E_T per unit sapwood area or ground area and in response to climatic conditions and this was suggested to be responsible for the differences in E_T between the plots. Growth or WUE were not examined but E_T per basal area, sapwood area or crown projection area often varied by >100% for each given species between treatments	Gebauer et al. (2012)
6	No, basal area of the all plots varied by <3%	<i>Quercus cerris</i> , <i>Quercus petraea</i>	Mediterranean (Italy). Mean annual precipitation of 950 mm	Forest. 50–60 years old. 689–933 trees ha ⁻¹	One mixture and a monoculture of each species ($n = 1$ for each treatment). Measured five dominant/codominant trees per species per treatment for a total of 16–20 days including four measurement periods	Drought reduced SFD of $Q.p$ by 52% in mixture and monoculture. Drought reduced SFD of $Q.c$ by 56% in mixture and only 31% in monoculture. This was consistent with increases in leaf $\delta^{13}C$ values of $Q.c$ in mixture and no change for $Q.p$ in mixture. The responses may have resulted from differing competitive abilities due to the shallower root system of $Q.c$. In addition, or alternatively, tree size might have influenced results because $Q.c$ trees were much larger in monoculture (Diameter, $D = 40$ cm, sapwood area = 802 cm ²) than in mixtures ($D = 25$ cm, sapwood area = 380 cm ²), whereas $Q.p$ were similar in monoculture ($D = 30$ cm, sapwood area = 210 cm ²) and mixture ($D = 27$ cm, sapwood area = 171 cm ²)	Grossiord et al. (2014b)

(Continued)

Table 1. Continued

Study	Allowed stand density to vary naturally?	Species	Climatic zone	Stand characteristics	Experimental design	Results	References
7	Yes	<i>Pinus sylvestris</i> L., <i>Picea abies</i> , <i>Betula pendula</i>	Boreal (Finland). Annual precipitation during study year was 700 mm. Basal area 1.7–24.4 m ² ha ⁻¹	Plantation. 12 years old. 1.5-m spacing (4444 trees ha ⁻¹)	Seven plots including the monocultures and each 2- and 3-species mixture ($n = 1$ for each species combination). Measured 3–5 dominant trees per species per treatment, each for 13 days in summer	The species diversity did not influence mean tree size for any species, total plot above-ground biomass or E_T . However, there was a high variability for each of these, and they were mainly influenced by the proportion of $B.p.$ For both tree biomass and E_T , $B.p.$ was highest and $P.a.$ was lowest. Mixtures containing $B.p.$ usually had higher E_T than expected from monocultures, perhaps due to the superior competitive ability of $B.p.$, but the statistical significance of treatment effects could not be tested	Grossiord et al. (2013, 2014a)
8	No, minimal differences in basal area in a given forest type	Sixteen European species	Boreal, Temperate, Mediterranean. Mean annual precipitation of 499 to 850 mm	Forest	160 plots in five major forests types of Europe. Used $\delta^{13}C$ of tree rings from plots with varying species combinations and diversity as a proxy for drought stress and changes in water availability	All species were affected by drought. The stand-level $\delta^{13}C$ declined with diversity in the two forest types that were more drought prone (temperate beech, thermophilous deciduous forests), indicating that diversity reduced the intensity of the drought stress. There was no relationship in the other three forest types (hemi-boreal, mountainous beech, Mediterranean forests)	Grossiord et al. (2014a, 2014c)

processes in forests are rarely examined in mixtures because they are generally very labour intensive, and as a result only one study in Table 1 measured more than five individuals per species per treatment for all species within the mixtures, and Studies 1 and 2 (in Table 1) were the only studies with replication at the plot level for each species combination.

Complementarity and transpiration in mixtures

The relationship between growth, transpiration and WUE can be expressed using the production ecology equation (Eq. (1), Binkley et al. 2004).

$$\begin{aligned} \text{Gross primary production} &= \text{resource supply} \\ &\times \text{fraction of resource acquired} \\ &\times \text{resource use efficiency} \quad (1) \end{aligned}$$

If the focus is on above-ground biomass growth (Mg ha⁻¹ year⁻¹), then this can be described using Eq. (1) as a function of the water supply (l ha⁻¹ year⁻¹), acquisition (a fraction) and WUE (Mg_{biomass} per litre of water transpired). Equation (1) was used in a review of the effects of species interactions on the nutrition of mixtures (Richards et al. 2010) and will also be referred to in this study.

Equation (1) has also been used in reviews that have shown that when tree or stand growth increases, there is generally also an increase in E_T and/or WUE (Binkley et al. 2004, Binkley 2012); there are usually no reductions in E_T or WUE when growth increases. This has been shown for growth responses to a wide range of treatments, usually in monocultures, including genetics, tree age, irrigation/drought, fertiliser application, pruning, thinning, species comparisons and geographic gradients (Binkley 2012, Forrester 2013).

With regard to mixtures, transpiration is one of the main processes that could influence water availability compared with monocultures and hence the susceptibility of mixtures to drought or their influence on local water supplies. Together, the studies in Table 1 indicate that at the stand and species levels, when there are no complementarity effects on growth, there are usually no complementarity effects on E_T or WUE. Here, complementarity is considered to occur when the mixture growth, E_T or WUE departs from the weighted average of monoculture values, which results when the interactions between species have a net positive (or negative) influence due to resource partitioning or facilitation (Loreau and Hector 2001). Studies 3, 5 and 7 in Table 1 are examples where there were no clear changes in growth, and also no stand-level changes in E_T and/or WUE. In those stands, the growth, E_T or WUE of the mixtures was a function of the properties of each species monoculture and the proportion of stand basal area, sapwood area or stand crown projection area that each species contributed to the mixture.

In contrast, Studies 1 and 2 found complementarity effects on growth and E_T and/or WUE of at least one species in the mixture,

resulting in increases in total stand growth, E_T and/or WUE. Depending on the species composition and age, this resulted from complementarity effects on all species in the mixture or it resulted because one species performed better in the mixture at the expense of another.

It is also critical to note that E_T is only one of many processes that could influence the water availability or water stress of trees in mixtures compared with monocultures. Other processes include (i) inter-specific differences in the proportion of precipitation that is intercepted by the canopy (Schume et al. 2004, André et al. 2008, Augusto et al. in press), or in the E_T per unit crown projection area, (ii) inter-specific differences in phenology or physiology that reduce competition for resources during parts of the growing season (Roupsard et al. 1999, Moore et al. 2011, Schwendenmann et al. 2015), (iii) the use of different water sources with one species extracting water from different depths due to inter-specific differences in root distribution, architecture or activity or due to species interactions that change these (Schume et al. 2004, Schwendenmann et al. 2015), (iv) increased water storage if the O horizon becomes deeper, or alternatively more evaporation and runoff if the O horizon is harder to infiltrate (Schume et al. 2004; Ilek et al. 2015), (v) the combination of isohydric species, which close their stomata during earlier stages of drought to conserve water to reduce the risk of embolism, with anisohydric species that open their stomata for longer into the drought, (vi) hydraulic redistribution where roots of one species extract water from deep moist soil and release it into shallower drier soil, thereby increasing the water availability for associated species (Neumann and Cardon 2012, Prieto et al. 2012), (vii) via shared mycorrhizal networks, although this may be a small flux of limited benefit to trees (Simard et al. 2012), or (viii) improved environmental conditions within the canopy such as where transpiration from an overstorey tree or shrub reduces the vapour pressure deficit and hence facilitates an understorey plant (Saccone et al. 2009), although this appears to have received very little attention with respect to tree–tree interactions in forests (as opposed to tree–seedling or shrub–seedling interactions).

These processes can often occur simultaneously, and in opposite directions, so information about one process may not give a good indication of the net effects of all water-related interactions. For example, in monocultures and mixtures of *Fagus sylvatica* and *Picea abies*, the *F. sylvatica* (a deciduous deeper rooter) used more water per crown projection area, but this was compensated for by higher interception rates of *P. abies* (an evergreen shallower rooter) (Schume et al. 2004).

Growth, E_T or WUE could also increase in response to processes that improve light and nutrient availability or uptake, which then increases growth and E_T and/or WUE, and in several studies in Table 1, it was these light- or nutrient-related processes that were probably the main drivers of the increases in growth, E_T or WUE, more than processes that directly influence

water availability or use (Forrester et al. 2010, Kunert et al. 2012). These processes can increase WUE (expressed in terms of above-ground growth) by shifting the partitioning of carbon from below-ground to above-ground, or by increasing the availability or uptake of nutrients or light enabling the plants to increase photosynthesis and make more efficient use of their water.

Given the spatial and temporal variability in soil and canopy conditions within mixed-species forests (Canham et al. 1999, Schume et al. 2004, Boyden et al. 2012) and the likely between-tree variability in growth and transpiration that results, when mixed-species forests are more productive than monocultures at the species and total levels, it may not necessarily be all trees that grow faster than those in monocultures and that contribute to increased E_T or WUE. The first aim of this study is to determine whether only the individual trees that grow faster in mixtures have higher E_T or WUE, while all other trees have similar rates of growth, E_T and WUE to comparable sized trees in monocultures. If so, stands without a population of trees that grow faster in mixture may be unlikely to have any complementarity effects on E_T or WUE. This could mean that growth measurements, which are much easier and cheaper than E_T measurements, might provide a good initial indication about whether mixtures are likely to be using more water.

Stand density and complementarity

It is too simplistic to suggest that when trees grow faster in mixtures, and use more water, they will automatically become more susceptible to droughts. This is because in addition to transpiration, the other water-related processes mentioned above could also influence water availability, and these processes together with the species interactions that influence them, could be beneficial during periods of water stress. While any interactions that improve light, water or nutrient availability could increase growth, E_T and WUE, it is the water-related processes that are most likely to reduce water stress by reducing competition for water thereby enabling transpiration and photosynthesis to be maintained at higher levels in mixtures than in monocultures. However, there is likely to be a trade-off between increasing productivity (and hence E_T), but not increasing it so much that there is a large enough reduction in water availability that outweighs the complementarity effect on water stress during a drought period. This trade-off may require that there are minimal differences in stand density between the mixtures and monocultures. However, by definition, if a mixture is growing faster than a monoculture, it has probably also developed a higher stand density in terms of basal area, sapwood area, leaf area and biomass, and the mixture is likely to transpire more water (Law et al. 2002). If stand density and productivity are significantly greater in mixtures, then mixtures may use more water than monocultures, but if stand density is similar, the water-related processes and interactions mentioned above could help to reduce any water stress of

trees growing in mixtures. While mixtures and monocultures may often vary in density, density can also be similar in mixtures and monocultures when it is managed by thinning, as is common in many forests, or alternatively each species may simply grow at similar rates.

Several studies in Table 1 specifically selected mixed and monospecific forest plots with similar densities in terms of basal area or leaf area (Studies 4–6 and 8). One of these (Study 8) showed that in forest types with higher drought frequencies, the drought stress, which was quantified by measuring stable carbon isotopes signatures of latewood, decreased as tree-species diversity increased. Interestingly, increases in drought stress from moist to dry periods were highest in the forest types with the highest densities. As mentioned above, most studies in Table 1 were done at the stand level and did not examine whether complementarity effects on E_T or WUE were modified by stand density. Therefore, the second aim of this study was to examine the interaction between individual tree neighbourhood density (in terms of basal area) and the size of complementarity effects on growth, E_T and WUE.

Climate and complementarity

The size of the complementarity effects between a given pair of species is dynamic. It changes as resource availability or climatic conditions change along spatial gradients from site to site, and along temporal gradients in climatic conditions or as stands develop and trees age (He et al. 2013, Forrester 2014). The interactions that influence water availability or use will probably be more useful on dry sites or during dry periods (Forrester 2014) and so for a given pair of species, the relative size of complementarity effects may increase as water availability declines if the main interactions influence water availability, E_T or WUE (Lebourgeois et al. 2013, Pretzsch et al. 2013b, Forrester 2014). The third aim of this study was to examine whether the complementarity effects changed with season.

The objective of this study was to test the following hypotheses.

1. In stands that show complementarity, it is only the individual trees that grow faster in mixtures that have higher E_T or WUE, while all other trees do not.
2. The individual tree neighbourhood basal area influences the size of complementarity effects on growth, E_T and WUE.
3. The interactions between species in terms of the sizes of the complementarity effects on growth, E_T and WUE vary between seasons.

The study was done in 14-year-old mixtures and monocultures of *Eucalyptus globulus* and *Acacia mearnsii* where stand-level growth, E_T and WUE were shown to have increased in mixtures by 73, 17 and 43%, respectively, compared with the *E. globulus* monocultures, and even more compared with *A. mearnsii* monocultures

(256, 93 and 76%; Forrester et al. 2010). Since these results (and most others in Table 1) were all at the stand level they provided no information about the three hypotheses listed above. The experiment is a useful platform to test these hypotheses because previous studies in the same plots measured nitrogen fixation, nutrient cycling, light absorption, above- and below-ground carbon allocation, and crown and fine-root architectures (Bauhus et al. 2000, Forrester et al. 2005, 2006, 2007, 2012c). To separate the effects of species composition, stand density and tree size, an individual tree-level approach was used. While it is difficult to measure many of the water-related processes at the individual tree level, the radial profile of sap flow within the sapwood varies with canopy exposure and soil water availability and with the vertical distribution of leaf area and its transpiration (Čermák and Nadezhdina 1998, Ford et al. 2004, Lu et al. 2004, Nadezhdina et al. 2007, Poyatos et al. 2007, Forrester et al. 2012b). In this study, the radial profile of the sap flux density (SFD) was used to indicate how the environment might have differed for trees of different sizes growing in different neighbourhood basal areas and under varying climatic conditions.

Materials and methods

Study area and experimental design

The experiment is 5 km southeast of Cann River, Australia (37°35'S, 149°10'E) and is Study 2 in Table 1. The climate is temperate and the long-term annual precipitation is ~1009 mm, which is distributed evenly throughout the year. During the 1-year study period the precipitation was only 730 mm. The mean daily maximum temperature in January is 26 °C and the mean daily minimum temperature in July is 2 °C. The soils are Brown Kurosol (Dy5.21) or Brown Dermosol (Gn4.31) (Stace et al. 1968, Isbell 1998) with a sandy loam A, AB or A2 horizon, a sandy clay loam B1 and B21 horizon above a light-to-medium clay B22 horizon. Prior to the establishment of the experiment the site vegetation was a dry sclerophyll forest dominated by *Eucalyptus sieberi* L. Johnson, which was cleared in 1991. *Eucalyptus globulus* seedlings were planted in early July 1992 and *A. mearnsii* in early October 1992. In November 1992, 25 kg P ha⁻¹ was applied in the form of superphosphate. The seedlings were planted using a replacement series design including monocultures and 1 : 1 mixtures at a spacing of 2 × 3.3 m (1515 trees ha⁻¹). In the mixtures the two species were mixed within rows creating a checkerboard arrangement of the two species. The plots (23 × 28 m) were arranged in a randomized block design in three replicate blocks.

By the age of 15 years the mean number of trees per hectare was 1263 in *E. globulus* monocultures, 1420 in mixtures (669 *E. globulus* + 751 *A. mearnsii*) and 1319 in *A. mearnsii* monocultures (Forrester et al. 2010). At the same age, above-ground biomass (Mg ha⁻¹) was 66.6 in *E. globulus* monocultures, 123.2 in mixtures (58.3 *E. globulus* + 64.9 *A. mearnsii*) and 78.0 in

A. mearnsii monocultures (Forrester et al. 2010). Above-ground biomass increment ($\text{Mg ha}^{-1} \text{ year}^{-1}$) between age 14 and 15 years was 3.28 in *E. globulus* monocultures, 5.66 in mixtures (4.81 *E. globulus* + 0.85 *A. mearnsii*) and 1.65 in *A. mearnsii* monocultures (Forrester et al. 2010). Leaf area index was 1.39, 2.07 and 1.35 in the *E. globulus* monocultures, mixtures and *A. mearnsii* monocultures, respectively. Additional information about the stand is in Forrester et al. (2010).

Tree measurements and neighbourhood density

Stem diameter overbark at 1.3 m (D ; cm) was measured in June 2006 and June 2007 (ages 14 and 15 years). Sapwood area (A_s ; cm^2) was predicted from D using allometric equations developed from trees within this experiment by Forrester et al. (2010). These were developed from cores collected from three to four points around the stem of 63 *A. mearnsii* and 54 *E. globulus* trees including those selected for water-use measurements. The sapwood–heartwood boundary was identified by colour difference, light transmission or with the assistance of 0.1% aqueous methyl orange stain.

Sap flux density (SFD; $\text{cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$) was measured from June 2006 to June 2007 using the compensation heat pulse technique (Edwards and Warwick 1984, Olbrich 1991). A total of 18 trees per species per treatment (36 per species) were selected that matched the diameter distribution within the plots, but due to logger failures this was reduced to a total of 34 *E. globulus* (D range 8.9–22.0 cm) and 32 *A. mearnsii* (D range 6.7–15.5 cm). The SFD of two to four of these trees per species per treatment were measured for periods of ~2 months using Teflon thermistor probes and stainless steel heaters (Edwards Industries, Otaki, New Zealand) and CR10X data loggers (Campbell Scientific, Logan, UT, USA). In each tree, four pairs of thermistor probes were inserted to four different depths into the sapwood at different aspects. These depths were determined by dividing the sapwood area into four rings of equal area and inserting the probes into the centre of each ring (Hatton et al. 1990). The heat pulse velocity was recorded every 30 min and the SFD was calculated after correcting for the effects of probe separation, wound diameter and volume fractions of water and woody matrix in the sapwood. Whole-tree SFD estimates were calculated using a second-degree polynomial regression against implantation depth using each of the four point estimates, as well as an additional data point with zero SFD at the sapwood–heartwood boundary. This regression was used to calculate an integrated average. When probe failures reduced the number of point measurements to <4, a weighted mean calculation was used (Hatton et al. 1990, Morris et al. 2004). Tree WUE ($\text{mm}^2 \text{ l}^{-1}$) was calculated as the mean daily growth ($\text{mm}^2 \text{ day}^{-1}$) of that tree over the 1 year growth measurement period divided by the mean daily transpiration of that tree (l day^{-1}) calculated over the 2-month sap flow measurement period.

The density of each individual trees' neighbourhood was calculated as the sum of the basal area (cm^2) of all trees within a

radius of 5 m of the target tree at age 15 years. This radius was shown to be an optimal distance for maximizing the correlation between target tree growth and neighbourhood competition in this experiment (Forrester et al. 2011).

Data analyses

When comparing mixtures and monocultures it is pertinent to consider that differences in growth, E_T , WUE or other processes, between mixtures and monocultures can be divided into two components: a selection effect and a complementarity effect (Loreau and Hector 2001). Selection effects occur when species with particular traits dominate at the expense of others; it is quantified using the covariance between a monoculture yield and the relative yield of the given species in the mixture. In contrast, complementarity occurs when the mixture yield is a departure from the weighted average of monoculture yields due to interactions between species that have a net positive (or negative) influence. Loreau and Hector (2001) showed how to separate these effects at the total stand level. It is important to note that this calculation and the use of the replacement series design assume that simultaneous changes in species proportion with the density of a given species (as opposed to the whole stand) are not important. Alternative approaches to quantify complementarity are also available (Fox 2005) but are not used in this study. To understand the contributions of individual species and the processes driving them it is also useful to examine species-level responses and in this study complementarity is quantified for a given species using Eq. (2) (Forrester 2014).

$$\text{Complementarity (\%)} = \frac{X \text{ in mixture} - X \text{ in monoculture}}{X \text{ in monoculture}} \times 100 \quad (2)$$

where X is growth, E_T or WUE.

To test whether only the individual trees that grow faster in mixtures have higher E_T and WUE, the trees in the mixtures were divided into two groups. The fastest growth rate of all *E. globulus* trees in monoculture was $5.7 \text{ mm}^2 \text{ day}^{-1}$. In the mixtures, any trees that grew at this rate or lower were considered slow growing and labelled as 'Mixture—slow' ($n = 10$) while all other trees in the mixture were labelled as 'Mixture—fast' ($n = 6$). The same grouping could not be done for the *A. mearnsii* because by the age of 14 years the ranges of individual tree growth rates were similar in mixtures and monocultures, even though trees in mixtures had been growing significantly faster prior to this study and still had larger diameters in mixture (Table 1; Forrester et al. 2011).

The effects of tree size (D), neighbourhood basal area and climate on the radial profile of SFD was examined using the radial profiles and climatic data that were measured at 14:30 h, because this was generally the time with peak SFD. Due to a weather station logger failure there was no half-hourly climate

data for the sixth 2-month measurement period so this was not included in the analysis. The climatic variables examined included precipitation, vapour pressure deficit and mean maximum and minimum temperatures.

Hierarchical mixed-effects models with R 3.0.2 (R Core Team 2013) were used to examine the effect of D , treatment and neighbourhood basal area on individual tree basal area growth, E_T and WUE. Random effects of measurement period nested within replicate were included to account for the hierarchical structure of the dataset. The effect of treatment, D and climate on the relationship between SFD and the radial depth into the sapwood was also examined using hierarchical mixed-effects models, with random effects of tree nested within replicate. A radial depth-squared term was also included to obtain the shape of the relationship. Initially all fixed-effect variables and two-way interactions were included in the models before all non-significant ($P > 0.05$) variables were removed in order of decreasing P -value. When none of the random effects were significant, linear models were fitted using least squares. Linear models were also used to examine relationships between complementarity (Eq. 2) and the climate of each measurement period. Each response variable was log transformed to remove heteroscedasticity of residuals and/or to linearize relationships. In the SFD models, serial autocorrelation resulting from the repeated measurement of SFD was accounted for by including a first-order autoregressive correlation structure with the same nested structure as used for the random effects.

Results

Complementarity and transpiration in mixtures

Growth and E_T of *E. globulus* trees increased with tree diameter (Figure 1). The diameter– E_T relationship was the same in mixtures and monocultures; however, the relationships between diameter and growth or WUE were significantly different such that trees of a given diameter in mixtures had faster growth and WUE than trees in monocultures. Consistent with the first hypothesis, it was only the trees that grew faster in mixtures that had the greater WUE in the mixtures; there was negligible difference in these relationships for trees in mixtures that had similar growth rates to those in monocultures. For *A. mearnsii* trees the relationships between tree diameter and growth, E_T or WUE were often insignificant (see Figure S1 available as Supplementary Data at [Tree Physiology](#) Online). In contrast to the *E. globulus*, the mean growth rates of *A. mearnsii* trees were lower in mixtures and this was associated with a reduction in E_T and WUE (Table 1).

Density and complementarity

There was a wide range in neighbourhood basal areas within the plots even though they were all planted at 1515 trees ha⁻¹ (Figure 2). The mortality rates were low in these stands and

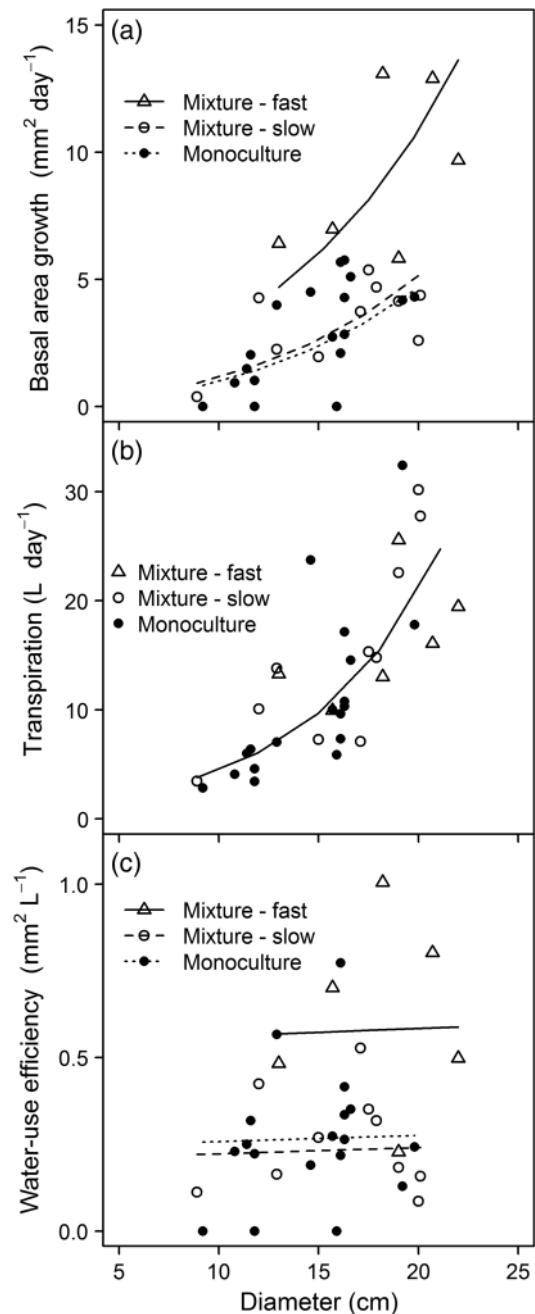


Figure 1. The relationship between diameter and basal area growth (a), transpiration (E_T ; b) and water-use efficiency (c) of *E. globulus* trees in mixtures and monocultures. In mixtures the trees are divided into trees that grew faster than all of the *E. globulus* trees in the monoculture (Mixture—fast) or trees that grew slower than the fastest growing *E. globulus* tree in the monocultures (Mixture—slow). The fit statistics are provided in Table S1 available as Supplementary Data at [Tree Physiology](#) Online.

survival rates were still >90% at the age of 11 years (Forrester et al. 2004) so the wide range in basal areas reflects differences in tree sizes more than tree numbers. The neighbourhood basal areas of *A. mearnsii* trees in mixtures were usually larger than those of the trees in monocultures. In contrast, there was a much larger overlap in neighbourhood basal areas of *E. globulus* trees in mixtures and monocultures, such that about half of the trees

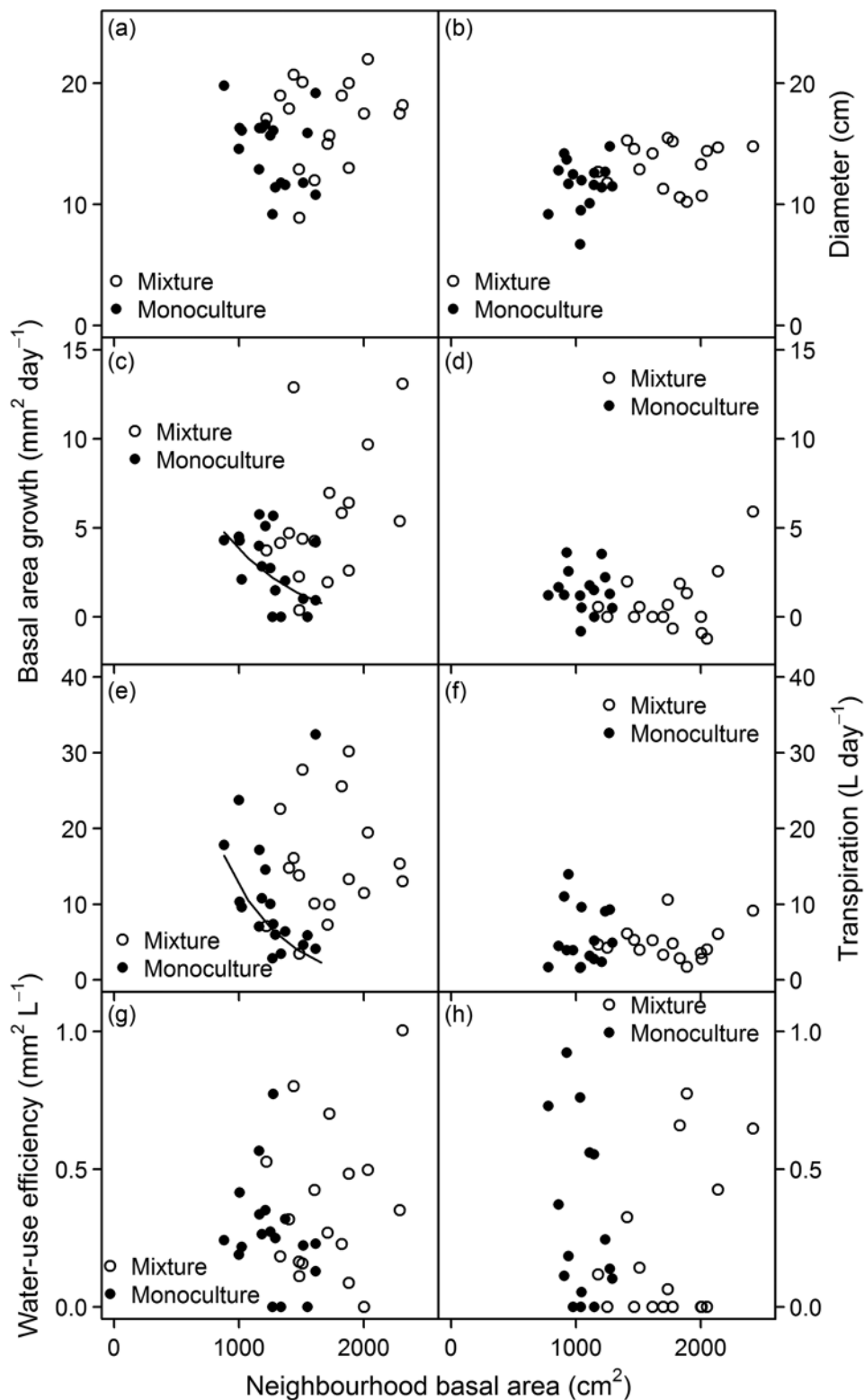


Figure 2. The relationship between neighbourhood basal area and diameter (D) (a and b), basal area growth (c and d), transpiration (E_T ; e and f) and water-use efficiency (g and h) of *E. globulus* trees (a, c, e and g) or *A. mearnsii* trees (b, d, f and h) in mixtures and monocultures. The fit statistics for the Monoculture lines in (c) and (e) are provided in Table S1 available as Supplementary Data at [Tree Physiology Online](https://academic.oup.com/treephys/article/35/3/289/1648522). For (e) the monoculture line is fitted without the outlier with a transpiration of 32 l day⁻¹.

growing in mixtures were growing at densities that were as low as those in monocultures, and about half of the trees in monocultures were growing at densities that were as high as those in the mixtures (Figure 2). It was therefore possible to examine the relationship between neighbourhood basal areas and the D , growth, E_T and WUE of the *E. globulus* trees.

There was no significant relationship between neighbourhood basal area and D in mixture or monoculture ($P = 0.15$). As expected, the relationship between neighbourhood basal areas and growth or E_T was negative in the monocultures in response to the increase in competition associated with an increase in neighbourhood basal area (Figure 2). In contrast, the same relationship was not significant in the mixtures ($P = 0.41$), and reflects the fact that in mixtures increasing neighbourhood basal area means an increase in the intensity of complementary interactions as well as competition. When only considering *E. globulus* trees in mixtures and monocultures that were growing at comparable neighbourhood basal areas, complementarity (Eq. (2)) was 141% (growth), 65% (E_T) or 54% (WUE). In contrast, when all trees were used, which represents a more representative sample from these stands, the complementarity effect was lower at 96% (growth), 45% (E_T) or 49% (WUE).

Climate and complementarity

The complementarity in terms of E_T for *E. globulus* varied from -30% to 191% between each of the six 2-month measurement periods and this was not related to the climatic conditions during each measurement period ($P > 0.32$). There was also no relationship for *A. mearnsii* or for either species when complementarity was calculated in absolute terms (without the denominator or $\times 100$ in Eq. (2); $P > 0.07$). This seasonal variability is consistent with stand-level results reported by Forrester et al. (2010) where the ranking of mixtures and monocultures changed throughout the year. This was not due to any confounding of measurement period with tree size or neighbourhood basal area, because both were similar during each measurement period (see Figure S2 available as Supplementary Data at [Tree Physiology Online](#)).

Radial SFD profiles

The radial SFD profile of both species was significantly affected by tree size, neighbourhood basal area, vapour pressure deficit and solar radiation (Tables 2 and 3). The SFD was higher in mixtures than in monocultures (Figure 3). The shape of the radial profiles also differed between mixtures and monocultures. For *E. globulus* it peaked near the cambium in monocultures whereas in mixtures the peak occurred at ~25% of the sapwood depth. The peak SFD also occurred deeper into the sapwood for *A. mearnsii* trees in mixtures compared with monocultures.

When considering the mean daily SFD there was no significant effect of tree size (Forrester et al. 2010). However, the SFD at

Table 2. Relationships describing the SFD at 14:30 h ($\text{ml cm}^{-2} \text{h}^{-1}$) as a function of proportional depth into the sapwood (Depth, 0–1), mixture/monoculture, diameter (D , cm), neighbourhood basal area (Nb, cm^2), vapour pressure deficit (VPD, kPa) and solar radiation (MJ m^{-2}). For all parameters $P < 0.05$. The random effects are shown in Table 3. SE, standard error.

Parameter	<i>A. mearnsii</i>		<i>E. globulus</i>	
	Estimate	SE	Estimate	SE
Intercept	1.67	0.39	-0.76	0.48
Depth mixture	7.4	0.83	4.8	0.33
Depth monoculture ¹	-2.73	0.36	-0.83	0.09
Depth-squared mixture	-5.49	0.79	-1.21	0.16
Depth-squared monoculture ¹	1.65	0.35		
D	0.039	0.022	0.196	0.038
VPD	-0.14	0.03	-0.01	0.03
Solar radiation	0.00102	0.00009	0.00048	0.00007
Depth $\times D$			-0.26	0.01
Depth \times VPD			-0.08	0.02
Depth \times solar radiation	-0.00087	0.00016	-0.00045	0.00013
Depth \times Nb	-0.00338	0.00047	-0.00021	0.00011
Depth-squared \times VPD	0.09	0.03		
Depth-squared \times Nb	0.0021	0.00045		
$D \times$ VPD	0.00666	0.00182	0.00774	0.00227

¹The mixture is used as the base level in the model, so to use the model for predictions, the parameter estimates from the monocultures need to be added to the parameter estimate for the mixture.

Table 3. Random effects for the models shown in Table 2. SD, standard deviation for the random effects; ρ , autocorrelation coefficient; e_{ij} , error component.

	<i>A. mearnsii</i>			<i>E. globulus</i>		
	Lower	Estimate	Upper	Lower	Estimate	Upper
SD (Replicate, i)				0.05	0.18	0.68
SD (Period, j)	0.33	0.42	0.55	0.31	0.41	0.55
ρ				0.55		
e_{ij}				0.57		

14:30 h, which was usually the time when it was highest, was found to significantly change with tree diameter when neighbourhood basal area, climate and depth into the sapwood were also accounted for (Figure 3). For *E. globulus* a reduction in tree diameter reduced SFD along the entire radial profile. For *A. mearnsii* a reduction in tree diameter reduced the slope of the relationship.

Neighbourhood basal area had a significant but minor effect on the SFD profiles of *A. mearnsii* (Figure 4). For *E. globulus* the effect of neighbourhood basal area was much greater, significantly increasing the SFD and moving the peak in SFD deeper into the sapwood as the neighbourhood basal area increased. The solar radiation and vapour pressure deficit also significantly influenced the radial profiles of each species; however, these effects were usually minor (Figure 5).

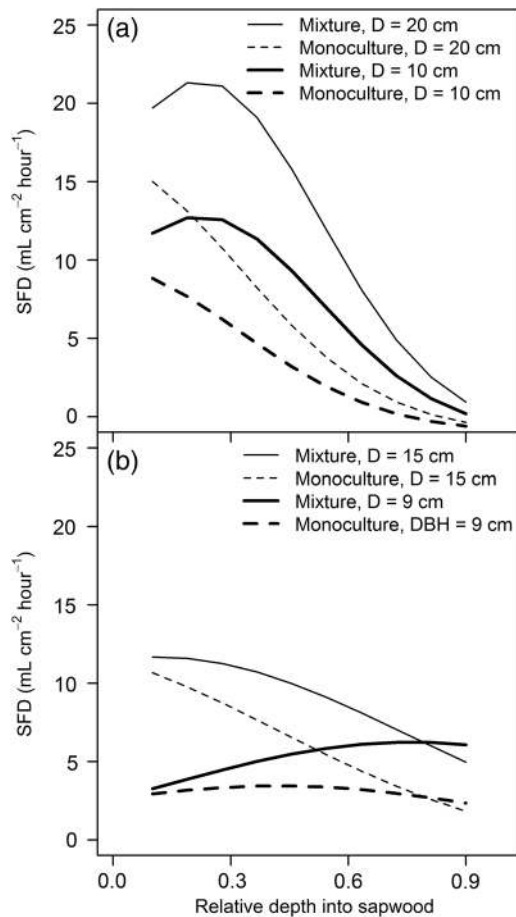


Figure 3. The effect of diameter (D) and mixing on the radial profile of sap flux density (SFD) for *E. globulus* (a) and *A. mearnsii* trees (b). Profiles are for 14:30 h, which was on average the time of peak SFD. The x-axis shows proportional implantation depth into the sapwood from the cambium; cambium = 0, sapwood-to-heartwood boundary = 1. The lines are fitted for average conditions with vapour pressure deficit of 1.5 kPa, solar radiation of 400 MJ m⁻² and neighbourhood basal area of 1470 cm². The fit statistics are provided in Tables 2 and 3.

Discussion

Complementarity and transpiration in mixtures

Stand-level investigations have shown that E_T and/or WUE increase in some mixed-species forests but not in others (Table 1). This study shows that even within a single stand, E_T or WUE can increase for some of the trees, but not for others. The responses of individual trees depend on their size and the species composition or basal area of their neighbourhoods. The stand-level patterns simply reflect the mean response. In this study, individual tree WUE was higher for *E. globulus* in mixtures than in monocultures, but it was only higher for the subset of trees that grew faster in the mixtures. This tree-level response is consistent with the stand-level responses at the individual species levels or the total stand levels in Table 1, and with a general pattern that when tree or stand growth increases, there is generally also an increase in E_T and/or WUE (Binkley et al. 2004,

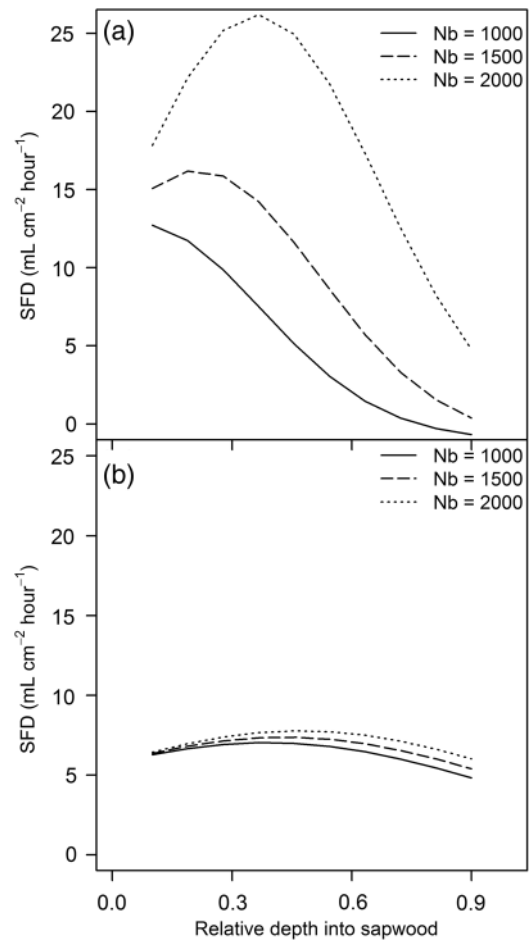


Figure 4. The effect of neighbourhood basal area (N_b ; cm²) on the radial profile of sap flux density (SFD) for *E. globulus* (a) and *A. mearnsii* trees (b). Profiles are for 14:30 h, which was on average the time of peak SFD. The x-axis shows proportional implantation depth into the sapwood from the cambium; cambium = 0, sapwood-to-heartwood boundary = 1. The lines are fitted for average conditions at vapour pressure deficit of 1.5 kPa, solar radiation of 400 MJ m⁻² and diameter of 15 cm for *E. globulus* or 12 cm for *A. mearnsii*. The fit statistics are provided in Tables 2 and 3.

Binkley 2012). Interestingly, there are usually no reductions in E_T or WUE when growth increases. The mixture studies in Table 1 show similar patterns such that E_T and/or WUE only increase in the mixtures when growth also increases. However, there is one exception in Table 1 (Study 1), where growth increased in five-species mixtures but WUE was lower than three-species mixtures (but still higher than monocultures). Since the results are presented at the total stand level it is not possible to see which species contributed to the reduction. The stand WUE declined mainly due to a decline in growth and since water was not limiting at that site, it was suggested that nutrients or light may have limited growth for periods during the growing season when atmospheric demand for water remained high. If this is indeed an exception, and E_T and/or WUE are usually only higher in mixture when there is also an increase in growth (for a given tree size and species), then measurements of growth would be a

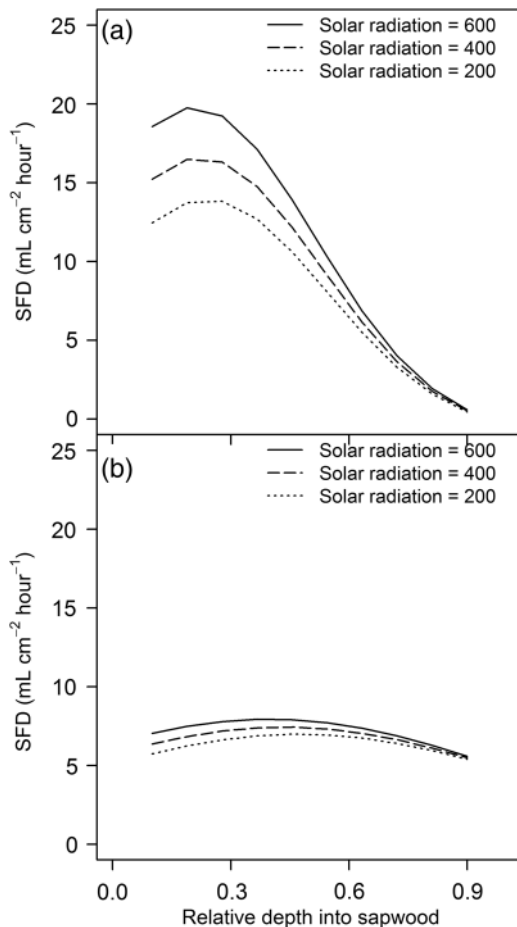


Figure 5. The effect of solar radiation (MJ cm^{-2}) on the radial profile of sap flux density (SFD) for *E. globulus* (a) and *A. mearnsii* trees (b). Profiles are for 14:30 h, which was on average the time of peak SFD. The x -axis shows proportional implantation depth into the sapwood from the cambium; cambium = 0, sapwood-to-heartwood boundary = 1. The lines are fitted for average conditions at vapour pressure deficit of 1.5 kPa, neighbourhood basal area of 1470 cm^2 and diameter of 15 cm for *E. globulus* or 12 cm for *A. mearnsii*. The fit statistics are provided in Tables 2 and 3.

cheap and simple way to predict whether mixtures are likely to have higher E_T or WUE than mixtures, and which tree sizes and species contribute to this effect.

The likely processes driving the complementarity of E_T and WUE in the mixtures were reflected in the radial profiles of SFD. It is assumed that outer sapwood is used more by higher branches or lateral roots, while the inner sapwood is used more by the lower branches and deeper roots (Dye et al. 1991, Lu et al. 2004, Nadezhdina et al. 2007, Fiora and Cescatti 2008, Forrester et al. 2012b), and therefore that the radial SFD profiles indicate the vertical distributions of both water uptake by roots and transpiration by leaves. Furthermore, because this site is not dry (even during the drought) and growth is restricted by nutrient limitations, it is assumed that the SFD profiles are a stronger reflection of the vertical distribution in leaf transpiration than in the vertical distribution of water uptake by roots. The

faster growth of *E. globulus* in mixtures largely resulted from improved nutrition via N fixation and accelerated rates of N and P cycling (Forrester et al. 2005, 2007). This was reflected in the radial SFD profiles of *E. globulus* trees in mixtures, which had a higher SFD and also a peak in SFD that was deeper into the sapwood. A shift in the peak SFD away from the cambium also resulted from N fertiliser application of *Eucalyptus nitens* trees in unthinned plantations. This was because the fertiliser resulted in an increase in photosynthesis and leaf area more in the mid- and lower-crown zones (Forrester et al. 2012a, 2012b). Furthermore, the crowns of *E. globulus* in the mixtures were generally higher than the neighbouring *A. mearnsii* trees and were probably exposed to higher light levels, similar to the situation trees might experience in thinned stands. The higher SFD across the entire sapwood radius in the mixtures is consistent with responses to thinning in *E. nitens* plantations (Forrester et al. 2012b). Since the crown architecture of *E. globulus*, in terms of length and width, did not change significantly between mixtures and monocultures at the age of 9.5 years (Bauhus et al. 2004), the contrasting radial SFD profiles may represent physiological changes. This is consistent with higher rates of photosynthesis and a shift in C partitioning more to above-ground in the mixtures (Forrester et al. 2006, 2012c).

The simpler SFD profiles of *E. globulus* in monocultures, where SFD declined almost linearly from the cambium to the heartwood boundary, may reflect a simpler vertical gradient in light, vapour pressure deficit and other microclimatic variables within the canopy in the absence of the *A. mearnsii* crowns and the lower range in neighbourhood basal area.

It is not clear what distinguishes the faster and slower growing trees in the mixtures. Most measurements of resource availability were made at the species or whole-stand levels but given the large spatial variability in light, water and nutrients at the scale of metres in forests (Canham et al. 1999, Schume et al. 2004, Boyden et al. 2012), it is likely that there is significant spatial variability within the mixtures. This variability probably results in a gradient in growing conditions with some trees benefiting more than others from the mixture with *A. mearnsii*.

In contrast to the *E. globulus*, *A. mearnsii* were growing more slowly in the mixtures where they had lower WUE. However, this clearly does not reflect the earlier growth dynamics because by ~11 years the basal areas of *A. mearnsii* trees in mixtures were 58% larger (Forrester et al. 2004). Furthermore, the strong facilitative effect of the *A. mearnsii* is still clearly influencing the *E. globulus*. The radial SFD profiles of *A. mearnsii* also reflect the crown architecture that developed during these earlier stages of development. In the mixtures the *A. mearnsii* have much deeper and wider crowns (Bauhus et al. 2004) and the higher SFD, especially within the inner sapwood, suggests that there are still higher rates of transpiration in these lower crown zones in the mixtures. In contrast there was a much smaller difference in SFD of the outer sapwood, which is consistent with no significant

differences in rates of photosynthesis in the upper crowns at the age of 15 years. Previously, the *A. mearnsii* and *E. globulus* had similar growth rates and complementarity interactions resulted in faster growth for both species in the mixture. However, the *A. mearnsii* are now growing much slower than the *E. globulus* (especially in the mixtures) so the balance between complementarity interactions and competition has shifted out of favour for the *A. mearnsii*. Their growth and WUE are probably lower in mixtures due to competition for light, water and nutrients, and possibly also because despite their slower growth, they are still larger in the mixtures and could therefore be closer to senescing after having progressed more rapidly through their life cycle.

Density and complementarity

Very few studies have examined the interactions between complementarity and stand density, especially with regard to processes other than growth. In terms of growth responses, the direction of the interaction varies between forests, with higher densities (in terms of biomass, basal area or trees per hectare) increasing complementarity in some forests (Boyden et al. 2005, Condés et al. 2013, Forrester et al. 2013) but decreasing it in others (Garber and Maguire 2004, Boyden et al. 2005, Río and Sterba 2009). The complementarity effects on *E. globulus* growth, E_T and WUE were all modified by neighbourhood basal area. The complementarity effects on growth and E_T were larger when calculated only using the trees in mixture and monoculture that were growing within the same range of neighbourhood

basal area (Table 4). This was because the relationships between neighbourhood basal area and growth or E_T were different in mixtures compared with monocultures. In monocultures, growth or E_T declined as density increased, as expected. However, in the mixtures the relationship was much weaker and not significant because as neighbourhood basal area increases so does the intensity of the interactions, and in mixtures, some of the interactions are complementary. The complementarity effects were greater when examined at the same density because this included the trees with the lowest growth or E_T in monocultures. The radial SFD profiles of *E. globulus* were higher and peaked deeper into the sapwood as neighbourhood basal area increased. This is a similar response to the effect of mixing on the SFD profiles and probably results from the same processes. As neighbourhood basal area increased, the mixing effect must also have increased and there were probably more intimate interactions with *A. mearnsii*.

The direction of the complementarity–density interaction probably depends on the types of interactions that occur between the species (e.g., water-related, light-related) and how changes in density influence the availability of those resources and the interactions. Therefore the interactions between density and complementarity in terms of growth, E_T or WUE are likely to differ between forests. Controlling stand density is an important silvicultural treatment used in forests to manage water availability (Hawthorne et al. 2013) and therefore understanding the interaction between complementarity and stand density may become increasingly important.

Table 4. Means and standard deviations of tree size, growth, transpiration (E_T), water-use efficiency (WUE) and neighbourhood basal area of *E. globulus* and *A. mearnsii* trees in mixtures and monocultures.

	<i>E. globulus</i>	Mixture		<i>A. mearnsii</i>
	monoculture	<i>E. globulus</i>	<i>A. mearnsii</i>	monoculture
All data				
Diameter (cm)	14.6 (2.9)	16.8 (3.5)	13.3 (1.8)	11.7 (2)
Sapwood depth (cm)	2.4 (0.4)	2.5 (0.3)	1.9 (0.2)	1.8 (0.2)
Sapwood area (cm ²)	74.2 (21.9)	94.0 (26.7)	61.8 (14.3)	52.3 (13.8)
Basal area growth (mm ² day ⁻¹)	2.8 (1.9)	5.5 (3.5)	0.8 (1.7)	1.5 (1.2)
E_T (l day ⁻¹)	10.8 (7.6)	15.6 (7.5)	4.9 (2.2)	5.5 (3.7)
WUE (mm ² l ⁻¹)	0.3 (0.2)	0.4 (0.3)	0.2 (0.3)	0.4 (0.4)
Neighbourhood basal area (cm ²)	1263 (208)	1697 (314)	1750 (326)	1058 (148)
Only <i>E. globulus</i> trees with intermediate neighbourhood basal area¹				
Diameter (cm)	13.6 (3)	16.1 (4)		
Sapwood depth (cm)	2.3 (0.4)	2.5 (0.3)		
Sapwood area (cm ²)	63.2 (21.9)	87.3 (26.7)		
Basal area growth (mm ² day ⁻¹)	1.9 (1.9)	4.6 (3.4)		
E_T (l day ⁻¹)	8.8 (8.6)	14.4 (7.4)		
WUE (mm ² l ⁻¹)	0.2 (0.2)	0.3 (0.2)		
Neighbourhood basal area (cm ²)	1387 (130)	1435 (110)		

¹The neighbourhood basal area was controlled by considering that the neighbourhood basal area for *E. globulus* trees growing in monoculture ranged from 881 to 1616 cm², while those for trees in mixtures ranged from 1222 to 2312 cm². These ranges overlap between 1222 and 1616 cm², and so any trees with neighbourhood basal areas within this range were used to calculate the means in the bottom half of the table. A similar grouping could not be done for the *A. mearnsii* because there was very little overlap in neighbourhood basal area between mixtures and monocultures (Figure 2).

Furthermore, when mixtures are more productive than monocultures they develop larger stand densities and so experimental designs that allow this density effect to be separated from the species composition effects are very useful. When this is not possible at the stand level, it is possible to use tree-level analyses and neighbourhood indices as in this study and others that have used neighbourhood indices to examine the complementarity effects of nutrient- and light-related interactions (Boyden et al. 2005, Forrester et al. 2013, Forrester and Albrecht 2014). Some studies select forest plots so that mixtures and monocultures have similar densities (Baeten et al. 2013) specifically to remove the confounding effects of stand density, as opposed to planted experiments where stands are usually left to develop without further manipulation (Kelty and Cameron 1995) and forest studies where plots are chosen that are fully stocked (Pretzsch et al. 2013a). However, when complementarity effects would naturally have resulted in faster growth rates and higher densities, then selecting same-density mixtures and monocultures (e.g., some studies in Table 1) may result in the selection of mixed-species plots where this complementarity effect has been thinned away by management or did not occur due to the climatic or edaphic conditions.

Climate and complementarity

There was clearly large intra-annual variability in complementarity effects in terms of growth, E_T and WUE, but these were not related to climatic conditions. Peaks in radial SFD can shift deeper into the sapwood as soil moisture declines or vapour pressure deficit increases and relatively more water is forced through the inner sapwood (Phillips et al. 1996, Gebauer et al. 2008, Hernandez-Santana et al. 2013). In this study, vapour pressure deficit and solar radiation had a significant but relatively small influence on the radial SFD profile so it is unclear why the complementarity effects varied so much during the year. The seasonal variability in complementarity in terms of E_T has been shown in several of the studies in Table 1 that were conducted for 1 year or more, including reversals in the ranking of treatments at different times of the year. This reflects the variability in soil moisture and atmospheric demand, the seasonality in the contribution of different water-related processes, and the fact that different species can peak in E_T at different times during the growing season and this timing can also be influenced by species interactions (Schume et al. 2004, Forrester et al. 2010, Moore et al. 2011, Kunert et al. 2012). These patterns probably also change from year to year as stands develop and in response to inter-annual climatic variability. The seasonal variability in complementarity effects on E_T or WUE shows that studies that consider only a small portion of the growing season may not provide a good indication of the annual complementarity effects.

Conclusions

In conclusion, when there are no changes in the growth of a given species in mixture, compared with monoculture, it may be unlikely that there will be any change in transpiration and/or WUE for that species. Similarly, at the individual tree level, a single mixed-species stand may contain some trees, of a given species, that grow faster than trees in the respective monoculture, and other trees of that same species that grow at similar rates to those in monocultures. This study shows that it may only be those trees that grow faster in mixtures that also have higher E_T and/or WUE than trees in monocultures, and clearly the stand-level patterns will reflect the mean tree-level response. Changes in the relationship between tree size and growth may therefore provide a good initial indication about whether E_T or WUE for a given species are likely to vary between mixtures and monocultures, and which size classes are the most affected. In addition, the complementarity effects on E_T and WUE can also be influenced by stand density and can change significantly throughout the year. This has implications for experimental designs and the duration of measurement periods and suggests that measurements of water-related processes that run for at least one whole year or growing season are most valuable.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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