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REGULAR ARTICLE

Above- and below-ground interactions of grass and pasture legume species when grown together under drought and low phosphorus availability

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Abstract Interactions between annual grass and perennial legume species when they are grown together under drought and limited phosphorus (P) availability are likely to be very important for pasture productivity, but are not well understood. Therefore, the objective of this study was to compare the interactions of drought and species combination on growth, nutrition, hydraulic lift and photosynthesis of the Australian native legume *Cullen australasicum* and the exotic legume *Medicago sativa* when grown with the exotic annual ryegrass (*Lolium rigidum*) with poorly soluble $FePO_4$ as the source of P. Plants were grown for 22 weeks in monoculture and in legume-grass mixtures in 1-m tall pots filled with river sand. Two moisture treatments were applied, drought (top 70 cm of soil allowed to dry after 16 weeks of establishment) and control (field capacity). In monoculture, shoot dry weight (DW) pot^{-1} of *L. rigidum* was higher than that of *C. australasicum* and *M. sativa*. In the mixtures, compared with the monocultures, an increase in shoot DW pot^{-1} for *L. rigidum* and a decrease for both *C. australasicum* and *M. sativa* resulted in a relative yield total >1. Citrate was the main carboxylate in the rhizosphere of all species, except for the drought-treated *L. rigidum* in monoculture and mixtures, for which malate was the main constituent. Both *C. australasicum* and *M. sativa* had higher concentrations of Ca, Mg, S, Cu, Zn, Mn and Mo in their leaves than did *L. rigidum*. Hydraulic lift was not detected in *M. sativa* and *C. australasicum*; likely reasons are discussed. Photosynthetic rate was similar for all species, but *L. rigidum* had tighter stomatal control. *C. australasicum* survived longer under drought than did *M. sativa*. In conclusion, *L. rigidum* out-competed the legumes. The legumes provided benefits to the growth of *L. rigidum* through solubilising P, but not through hydraulic lift. In addition, *L. rigidum* conserved moisture through tight stomatal control and produced an extensive root system to take up water and nutrients efficiently.

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Introduction

In Australia, half of the total arable land area is regularly affected by drought (Smithson and Sanchez 2001) and this is expected to expand under predicted climate-change scenarios (Mpelasoka et al. 2008). In response to drought and to manage dryland salinity, which develops due to the hydrological imbalance that results when native, deep-rooted perennial vegetation is removed and replaced by shallow-rooted annual crops and pastures (Hatton and Nulsen 1999), Australian perennial legumes are now considered to have potential for development as pasture legumes (Dear et al. 2007; Robinson et al. 2007; Pang et al. 2010; Suriyagoda et al. 2010a). These species are likely to have evolved in local P-impoverished environments (Beadle 1966; Handreck 1997). In particular, *Cullen australasicum* (Schltdl.) J.W. Grime, appears well adapted to low-rainfall areas with acidic soils in the Western Australian wheatbelt (Li et al. 2008; Bennett et al. 2011). Incorporation of this native species into agricultural systems would result in greater plant diversity and, presumably, wider adaptation to diverse climatic and soil conditions (Cocks 2001; Bennett et al. 2011). However, the response of *C. australasicum* to drought and nutrient dynamics is largely unknown. Also, plant responses to drought and nutrient deficiencies may differ markedly when grown in mixed-pasture systems to when the same plants are grown individually or in a monoculture (Lucero et al. 1999, 2002). Only a few attempts have been made to study the interaction of pasture species grown in mixtures under Australian agricultural systems (Dear et al. 2007; Hayes et al. 2008) and there is little information on even widely grown pasture species such as annual rye grass (*Lolium rigidum* L.) and lucerne/alfalfa (*Medicago sativa* L.). One way to assess the competitiveness of these pasture species in mixtures is through a de Wit replacement series (Jolliffe 2000).

After nitrogen, phosphorus (P) is usually the most limiting nutrient for crop production (Schachtman et al. 1998). Phosphate concentrations in soil solution are often <10 μM (Raghothama 1999) and are subjected to rapid depletion due to absorption of P by roots when the rate of replenishment of soil solution P is slower than plant uptake of P (Claassen and Barber 1976; Silberbush and Barber 1983). The labile phosphate removed by plants is replaced by the mobilisation of less labile soil phosphate fractions in the soil. The concentration of phosphate in the soil solution is partly controlled by the solubility of sparingly soluble soil phosphates (e.g. Ca-P, Al-P, Fe-P) (Elrashidi and Larsen 1978). The solubility of phosphate minerals depends on the concentration of protons, P ions and either Ca, Fe or Al ions that can co-precipitate. Ca-phosphates dominate in neutral-alkaline soils, and have increasing solubility with decreasing pH. Conversely, Fe and Al-phosphates form in acid soils, and become increasingly soluble with increasing pH (Lindsay 1979; Bolan et al. 1987). Although many Western Australian soils are very low in total P in their native state, due to the extensive use of P fertiliser, substantial accumulation of sparingly soluble P has occurred (Bolland and Gilkes 1998).

Plants evolve different mechanisms to utilise relatively immobile, and often poorly available, P (e.g. $FePO_4$ in acidic soils) allowing them to respond to P deficiency (Vance et al. 2003; Raghothama and Karthikeyan 2005). These mechanisms increase in importance under drought (Henkin et al. 1998; Sardans and Peñuelas 2007). One mechanism to enhance P acquisition is to alter root physiology (Neumann and Martinoia 2002), such as by enhanced exudation of carboxylates (e.g., malate and citrate) and phosphohydrolases (Richardson et al. 2000; Wouterlood et al. 2005). Root exudates are also important in the maintenance of root-soil contact, which is especially important under drought and drying conditions when hydraulic continuity is lost (Young 1995; Walker et al. 2003). Even though some reports are available on carboxylate exudation by perennial ryegrass (*Lolium perenne* L.) (Rosas et al. 2007), wheat (*Triticum aestivum* L.) (Pearse et al. 2006) and several other grass species (Ryan et al. 2001 and references therein), information on *L. rigidum* is not yet available. Pang et al. (2010) studied the carboxylate exudation of *C. australasicum* and *M. sativa* under two [P]. They found only *C. australasicum* increased carboxylate exudation at low-P supply (6 mg kg^{-1}) compared with high-P supply (40 mg kg^{-1}). Furthermore, only Suriyagoda et al. (2010a) has examined carboxylate exudation in *C.*

australasicum and *M. sativa* under drought and these plants were grown in monoculture. Depending on the extent to which these exudates diffuse around roots, nutrient availability may change and affect nutrient uptake at a very local scale, that is, in the rhizosphere, or at the scale of individual plants and their neighbours (Raynaud et al. 2008). In the latter case, exuding plant species may behave as ecosystem engineers sensu Jones and Darrah (1994), because they allow an increase in the nutrient uptake of neighbouring plants. Such a mechanism may therefore have considerable effect in controlling the competition for soil resources and the dynamics of plant communities (Raynaud et al. 2008). In shoots, drought and P deficiency can reduce photosynthetic rate (A) and stomatal conductance (g_s) (Jacob and Lawlor 1991; Ghannoum and Conroy 2007) and thereby restrict plant growth.

Hydraulic lift is the passive movement of water from roots into soil layers with lower water potential, while other parts of the root system in moister soil layers, usually at depth, are absorbing water (Caldwell et al. 1998). The magnitude of hydraulic lift in perennial and annual field crop and pasture systems is not known, but if it does occur, this process of hydraulic lift could have significant implications for irrigation, fertilisation, and intercropping (Caldwell et al. 1998). Very few researchers have actually examined or discussed hydraulic lift in crop and pasture species (Corak et al. 1987; Vetterlein and Marschner 1993; Wan et al. 2000; Sanderson et al. 2004). In an agricultural field experiment, deep-rooted leguminous intercrops lifted water and some of this water was used by associated shallow-rooted crops that had no direct access to the deep water (Sekiya and Yano 2002). Skinner et al. (2004) investigated and discussed the occurrence of hydraulic lift in humid temperate pasture systems with *L. perenne*, orchard grass (cocksfoot) (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.) sown as grass/legume mixtures into field plots of which half included a deep-rooted perennial forb (chicory; *Cichorium intybus* L.). Under current climate-change scenarios, with the area of drought- and salinity-affected lands predicted to expand, incorporation of species with greater potential for hydraulic lift would enhance the sustainability of marginal agro-ecosystems.

Given our lack of information on Australian legumes with potential as pasture species, the objective of this study was to compare the effects of the interaction of water availability and species combination under conditions of poorly soluble P ($FePO_4$) availability on growth, nutrition and photosynthesis of the native legume *C. australasicum* and *L. rigidum*, an important annual pasture grass, and weed, of cropping systems, in southern Australia. The responses were compared with those of a widely cultivated exotic perennial legume, *M. sativa*. We hypothesised that: (i) plants in mixtures would grow better than those in monocultures, because (a) organic anions exuded from the legume may release 'extra' P for growth by both plant species and/or (b) deep rooted legumes would lift water from depth and release it in topsoil layers to support growth by the shallow rooted grass in drying soil, (ii) nutrients would be mobilised from senescing leaves for new growth, especially under moist soil conditions, and (iii) *C. australasicum* would tolerate drought better than *M. sativa*.

Materials and methods

Growth conditions

Cullen australasicum accession SA4966 (C), *M. sativa* cv. SARDI-10 (M) and *L. rigidum* Gaudin cv. Safeguard (L) were grown in two-plant combinations (i.e. CC, MM, LL, CL, ML) 5-cm apart, in 1-m tall, 15-cm diameter vertically split pots. Seeds of *C. australasicum* were collected from seed-multiplication plots established at the Shenton Park field station of the University of Western Australia. *M. sativa* seeds were sourced from the Genetic Resource Centre at the South Australian Research and Development Institute and *L. rigidum* seeds from the Western Australian Herbicide Resistance Initiative (WAHRI) at the University of Western Australia. The experiment consisted of two moisture treatments: control (watered from both top and bottom using a wick system throughout the experiment) and drought (watered from both top and bottom using a wick system until 16 weeks, and thereafter the top compartment was allowed to dry out, while the wick from the bottom remained active and transferred water up to 20 cm from the bottom of the pot). Seven replicate pots of each species×moisture combination were established. Pots were filled with 17 kg pot^{-1} of thoroughly washed, steam-sterilised

river sand. Basic available soil [P] was 1–2 mg P kg^{-1} dry sand and pH ($CaCl_2$) was 6.0, as determined by CSBP FutureFarm analytical laboratories, Bibra Lake, Australia. All essential nutrients except P were provided by amending the sand with 126.6 mg kg^{-1} $Ca(NO_3)_2.4H_2O$, 42.8 mg kg^{-1} NH_4NO_3, 178 mg kg^{-1} K_2SO_4, 101 mg kg^{-1} $MgSO_4.7H_2O$, 11 mg kg^{-1} $CaCl_2.2H_2O$, 12 mg kg^{-1} $MnSO_4.H_2O$, 8.8 mg kg^{-1} $ZnSO_4.7H_2O$, 1.96 mg kg^{-1} $CuSO_4.5H_2O$, 0.68 mg kg^{-1} H_3BO_3, 1.01 mg kg^{-1} $NaMoO_4.2H_2O$ and 32.9 mg kg^{-1} FeNaEDTA. P was supplied by amending the sand only in the top 30-cm of a pot with 100 mg kg^{-1} $FePO_4$ and 5 mg kg^{-1} KH_2PO_4. By amending the soil with KH_2PO_4 (i.e. a source of readily available P) initial establishment was ensured. *C. australasicum* and *M. sativa* seeds were mechanically scarified and imbibed to enhance the germination rate, before sowing in seedling trays at staggered times according to their pre-determined germination time. *L. rigidum* seeds were germinated 2 weeks after the planting of *C. australasicum* and *M. sativa*, in order to ensure a vigorous and uniform establishment of legumes and to minimise the initial competition. The experiment was set out in a glasshouse with pots 30 cm apart so as to avoid edge effects, at the University of Western Australia, Perth (31°59′S, 115°53′E), as a randomised complete block design. Three seedlings were planted in each pot and thinned to one plant of each species (two in the monocultures). Weekly additions of 300 mL of 2 mM NH_4NO_3 commenced at week six to ensure an adequate nitrogen supply. Pots were randomised within each block every 2 weeks. The glasshouse was unheated and had an average daily temperature of 20°C during the experiment, which was conducted from June to November 2009. Three replicates of each species combination were harvested at 16 weeks, just before the drought treatment began. Note that the *M. sativa* and *L. rigidum* plants exposed to the drought treatment reached their permanent wilting point 3–4 weeks after the drought treatment began. Therefore, those plants were harvested 4 weeks after the start of the drought treatment. For all other treatment combinations harvesting was done at 22 weeks (i.e. 6 weeks after the drought treatment began). Care was taken to collect all the leaves shed from each plant daily. Furthermore, due to the shedding of green leaves of *M. sativa* in the drought treatment, nutrient analysis of green leaves was not possible.

Physiological measurements

Volumetric water content (VWC-v/v) was measured at the beginning of the experiment and thereafter at 2–3 week intervals until the drought treatment began, after which measurements continued regularly at short time intervals until the end of the experiment. For that purpose 30-cm length three-rod metallic probes (CS605, Campbell Scientific Inc., Logan, Utah, USA) connected to a reflectometer (TDR 100, Campbell Scientific Inc., Logan, Utah, USA) were vertically placed in the top 30 cm of each pot between two plants. Measurements were made in the evenings (1,800–1,900 h) before the drought treatment began, and thereafter in the evenings and in the early morning of the following day (0,500–0,600 h). Due to the activity of the wick system and capillary rise, an~20-cm soil layer at the bottom of all pots was maintained at field capacity throughout the experiment. Leaf water potential (Ψ) was measured at midday (1,200–1,400 h) in a pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) on petioles of young fully expanded leaves of *C. australasicum* and *M. sativa*. For *L. rigidum*, pseudo-stems with several leaves were used. Measurements of Ψ were made two days before the drought treatment began and two days before the final harvest (at 22 weeks), except for drought-treated *M. sativa* and *L. rigidum* plants, for which measurements were taken 3 weeks after the drought treatment began.

Photosynthetic rate and g_s were measured between 1,000 and 1,400 h on the youngest, fully expanded leaf of all plants, using a portable gas-exchange system (LI6400 portable, LiCor Inc., Lincoln, NE, USA) equipped with a light source (6400-02B LED, LiCor). Measurements were taken 1 week before and 3 weeks after the drought treatment began, and 2–4 days before the final harvest (22 weeks), except for drought-treated *M. sativa* and *L. rigidum*, for which measurements were taken only until 3 weeks after the drought began. Photosynthetic photon flux density at the leaf surface was maintained at 1,500 μmol m^{-2} s^{-1} during the measurement of A and leaf temperature was maintained at 25°C. Ambient humidity of the incoming air to the leaf chamber was left at that of the glasshouse environment. A was measured when the ambient CO_2 concentration of the incoming gas stream was 380 μmol mol^{-1}, a value close to that during plant growth.

Plant analyses

Root systems were gently removed from the bulk soil and root depth was measured before the drought treatment began and during the final harvest. However, it was impossible to completely separate the root systems of the two plants in a pot. Therefore, carboxylates were collected from the rhizosphere of the two plants in a pot. Roots were shaken slightly to remove the excess soil and the remaining soil was defined as the rhizosphere soil. Root fraction from the top 30-cm sand layer was transferred to a 200-mL vial and washed in a measured amount of 0.2-mM $CaCl_2$ solution ranging from 20 to 150-mL. The root system was gently dunked in the solution until as much rhizosphere soil as possible was removed. Care was taken to minimise root damage. A subsample of the rhizosphere extract was then filtered using a 0.2-μm syringe filter into a 1-mL HPLC vial. The vial was acidified with one drop of concentrated phosphoric acid, placed in dry ice, and transferred to a −20°C freezer until HPLC analysis. Details of the rhizosphere carboxylate estimation are given in Suriyagoda et al. (2010a). The root system was then washed more thoroughly to remove any residual soil.

The root system dry weight (DW) per pot, dead (shed) leaf, green leaf, stem and total above-ground DW per plant were determined after drying at 60°C for 1 week. Dead and green leaves of each plant were ground separately in a steel ball mill. An approximately 100-mg subsample was taken and digested in nitric/perchloric acid and analysed using inductively coupled plasma (ICP) atomic absorption with a Perkin Elmer Optima 5300 DV optical emission spectrometer (OES; Shelton, CT, USA) for all the nutrients, except N.

Leaf [N] was determined by dry combustion (Nelson and Sommers 1996) using an elemental CN analyser (Elementar Analysensysteme GmbH, Hanau, Germany) and is expressed on DW basis ($[N]_{DW}$). The ratio of A and g_s was used to derive the photosynthetic water-use efficiency (*WUE*).

Statistical analyses

Data were subjected to 2-way analyses of variance (PROC GLM) in SAS/STAT software Version 9.1 (SAS Institute Inc., Cary, NC, USA 2003) to examine the impact of species combination, moisture and their interactions on response variables. No transformations were needed. Results of the statistical analyses are summarised in Table 1. Comparisons between means were made using Tukey's Honest Significant Difference procedure. Means are presented with S.E. and significance is expressed at $p<0.05$.

Results

Midday leaf Ψ before the drought treatment was imposed was always higher than −1 MPa and was not affected by the species combination treatment (Table 1; Fig. 1a). At harvest, the moisture treatment had a significant impact on leaf Ψ, whereas the species combination treatment did not (Table 1; Fig. 1b). Indeed, shoots of control plants maintained a higher leaf Ψ during the entire experiment and the values of leaf Ψ at harvest did not differ from those measured before the drought treatment commenced (Fig. 1b). Note that at harvest for *C. australasicum* and *M. sativa* grown with *L. rigidum*, all mature leaves were shed, and hence leaf Ψ could not be determined.

Root depth before the drought treatment was imposed was affected by the species, with roots of both *C. australasicum* and *M. sativa* very close to 1 m depth, while roots of *L. rigidum* were at 35–40 cm depth, irrespective of companion species (Table 1; Fig. 2a). Root depth at harvest was also affected by the species, but not by the moisture treatment (Table 1; Fig. 2a). At harvest, *L. rigidum* roots had reached 60 cm depth.

Volumetric water content in the top 30 cm soil layer before the drought treatment commenced was not affected by the species combination treatment and ranged from 8-13% (v/v) (Table 1, Fig. 2b). During the first ten days of the drought treatment, VWC of the drought-treated pots was greatly reduced and was less than 3% at harvest. Control pots maintained higher and uniform VWC throughout the experiment. At harvest, VWC of the control was higher than that of the drought treatment, but it was not affected by the species combination treatment (Table 1). There was no difference between the evening and following day morning measurements of VWC at any date in either control or drought treated pots. Irrespective of the moisture treatment and species combination, the bottom 20-cm layer of all pots was at field capacity due to the activity of the wick and capillary rise (data not shown).

Root DW per pot at harvest was affected by the species combination treatment and the moisture treatment (Table 1). Root DW of monocultures of *C. australasicum* and *M. sativa* did not differ between moisture treatments, while the monoculture of *L. rigidum* and mixtures containing *L. rigidum* had a higher root DW per pot under control moisture conditions (Fig. 3a). In the monocultures under control moisture conditions, root DW of *C. australasicum* and *M. sativa* was substantially lower than that of *L. rigidum*.

Dead leaf DW at harvest was affected by the species combination treatment, but not by the moisture treatment (Table 1). Both *C. australasicum* and *M. sativa* had a very low dead leaf DW per plant in both monoculture and mixtures compared with that of *L. rigidum* (Fig. 3b). For *L. rigidum*, dead leaf DW per plant was threefold higher in mixtures than in monoculture. Green leaf DW at harvest differed with species combination and moisture treatments (Table 1). For all species, green leaf DW under control moisture conditions was higher than that under the drought treatment (Fig. 3b). When comparing species, both *C. australasicum* and *M. sativa* had very low green leaf DW per plant in both monoculture and mixtures, irrespective of the moisture treatment; thus their green leaf DW was markedly less than that of *L. rigidum*. Furthermore, for both *C. australasicum* and *M. sativa*, dead and green leaf DW per plant in monoculture was 3- to 5-fold higher than that produced in mixtures (Fig. 3b), while the opposite occurred for *L. rigidum*, where both dead and green leaf DW per plant were 3- to 4-fold higher in mixtures than in monoculture. Also, for all species in the drought treatment, dead leaf DW was higher than green leaf DW.

Table 1 Significance of different sources of variability

Character	Source of variability			
	S	M	S×M	R^2
Leaf water potential (before drought)	n.s.	n.a.	n.a	0.57
Leaf water potential (at harvest)	n.s.	***	n.s.	0.84
Root depth (before drought)	***	n.a	n.a	0.99
Root depth (at harvest)	***	n.s.	n.s.	0.94
VWC (before drought)	n.s.	n.a	n.a	0.74
VWC (at harvest)	n.s.	***	n.s.	0.82
Dead leaf DW per plant	**	n.s.	n.s.	0.84
Green leaf DW per plant	***	***	***	0.83
Total above ground DW per pot	***	***	**	0.88
Root DW per pot	**	**	n.s.	0.59
Carboxylate concentration	n.s.	***	*	0.66
Carboxylate composition	*	*	*	0.54
[P] in green leaves	*	n.s.	n.s.	0.45
[P] in dead leaves	***	**	n.s.	0.56
PUE	*	n.s.	n.s.	0.41
[N] in green leaves	***	***	n.s.	0.77
[N] in dead leaves	***	***	*	0.91
A (before drought begins)	**	n.a	n.a	0.74
A (3 weeks after drought begins)	**	***	**	0.67
A (at final harvest)	n.s.	***	*	0.71
g_s (before drought begins)	*	n.a	n.a	0.62
g_s (at harvest)	*	***	*	0.63
WUE	**	*	*	0.54

Significant effects are indicated for species combinations (S), moisture treatment (M) and their interactions (n.a., not applicable; n.s., no significant difference; * $p<0.05$; ** $p<0.01$; *** $p<0.001$).

R^2 for the fitted full model is given

Total above-ground DW per pot at harvest was affected by the species combination treatment and the moisture treatment (Table 1). When comparing the growth of different species under control moisture conditions and drought, growth of *L. rigidum* (shallow-rooted) was greatly reduced under the drought treatment compared with that of *C. australasicum* and *M. sativa* (Fig. 3c). For both *C. australasicum* and *M. sativa*, total above-ground DW per pot was 2- to 4-fold higher in monoculture than in mixtures, irrespective of the moisture treatment. However, for *L. rigidum* the total above-ground DW per pot was 1- to 2-fold higher in mixtures than that for *L. rigidum* in monoculture, irrespective of the moisture treatment. This differential growth response of the grass and legume in mixtures resulted in a relative yield total (*RYT*; ratio of the total aboveground DW per pot in mixtures and monocultures) >1, and the response was greater under control moisture conditions than in the drought treatment (Fig. 3c).

Rhizosphere carboxylate concentrations at harvest showed a species combination treatment×moisture treatment interaction (Table 1). Drought-treated monocultures of *C. australasicum* and *M. sativa* had very low rhizosphere carboxylate concentrations compared with those under control moisture conditions (Fig. 4a). However, *L. rigidum* was not responsive and maintained very low rhizosphere

carboxylate concentrations, irrespective of the moisture treatment. Carboxylate concentrations in *C. australasicum* and *L. rigidum* mixtures did not differ between moisture treatments while those of the *M. sativa* and *L. rigidum* mixture were higher under the control moisture condition. Furthermore, in monoculture *M. sativa* had a higher concentration of carboxylates compared with *L. rigidum* under control moisture condition. Carboxylate composition varied between moisture treatments and among species combination treatments (Fig. 4b). Citrate was the main carboxylate in the control, irrespective of the species combination. Citrate remained the main constituent in drought-treated *C. australasicum* and *M. sativa*. However, the proportion of malic acid was reduced and replaced by other carboxylates, mainly fumaric acid. For drought-treated *L. rigidum* in monoculture and mixtures, the proportion of citrate was reduced, while that of malate increased to 68-83% and that of shikimic acid increased to 8-13%.

Green leaf [P] at harvest was affected by the species combination treatment, but not by the moisture treatment (Table 1). In monoculture, *C. australasicum* and *M. sativa* had a higher [P] in their green leaves than *L. rigidum* (Fig. 5a). Green leaf [P] did not differ between monoculture and mixtures for *C. australasicum* and *L. rigidum*, while green leaf [P] of *M. sativa* in mixtures could not be determined, due to leaf shedding. Dead leaf [P] at harvest was affected by the species combination treatment and the moisture treatment (Table 1). In monoculture, *M. sativa* had a higher dead leaf [P] than *L. rigidum*, irrespective of the moisture treatment (Fig. 5b). Under the drought treatment in mixtures, dead leaves of *C. australasicum* and *M. sativa* accumulated a higher [P]; higher than that under the control moisture condition as well as higher than that of *L. rigidum*.

PUE at harvest, expressed in terms of leaf dry weight (both green and dead leaves) per unit of P taken up (g mg^{-1}), was affected by the species combination treatment, but not moisture treatment (Table 1). In monoculture, *L. rigidum* had a higher *PUE* than *C. australasicum* and *M. sativa* (Fig. 5c). However, in mixtures *PUE* of *L. rigidum* was higher only when it was grown with *M. sativa*.

Green leaf [N] at harvest was affected by the species combination treatment and moisture treatment (Table 1), due to *C. australasicum* plants in the drought treatment having very high [N]. Apart from that, no differences in green leaf [N] were present.

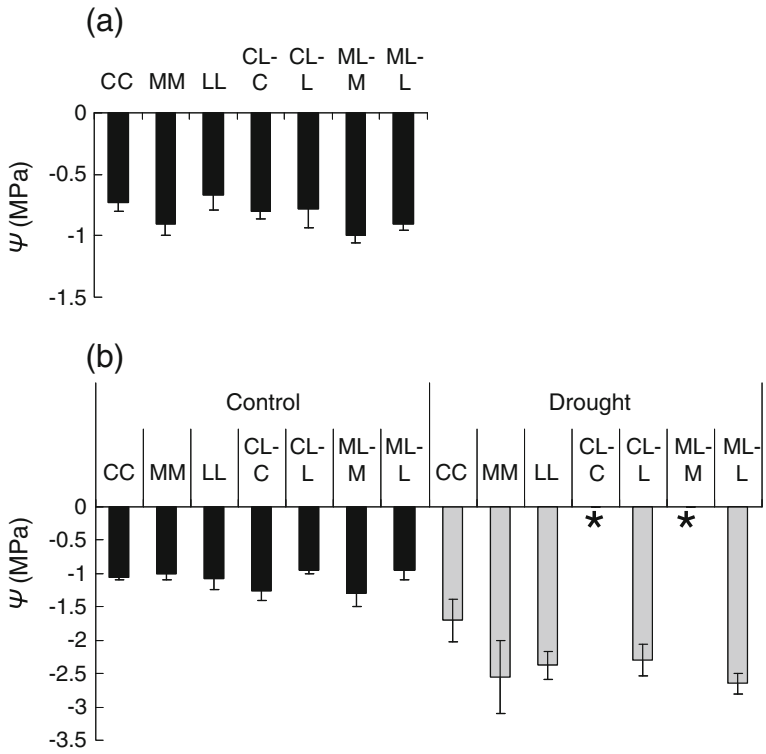

Fig. 1 Mid-day leaf water potential (Ψ) **a** before the drought treatment was imposed and **b** at the final harvest for monoculture *C. australasicum* (CC), *M. sativa* (MM) and *L. rigidum* (LL) and for mixtures of *C. australasicum* grown with *L. rigidum* (CL-C), *L. rigidum* grown with *C. australasicum* (CL-L), *M. sativa* grown with *L. rigidum* (ML-M) and *L. rigidum* grown with *M. sativa* (ML-L) under moist soil (*Control*) and top soil dry (*Drought*) treatments. * indicates that no large leaves were present at harvest to measure Ψ. Note that Ψ of drought treated MM, LL, CL-L and ML-L was taken 3 weeks after drought treatment began due to the early plant death (mean±S. E., n=4)

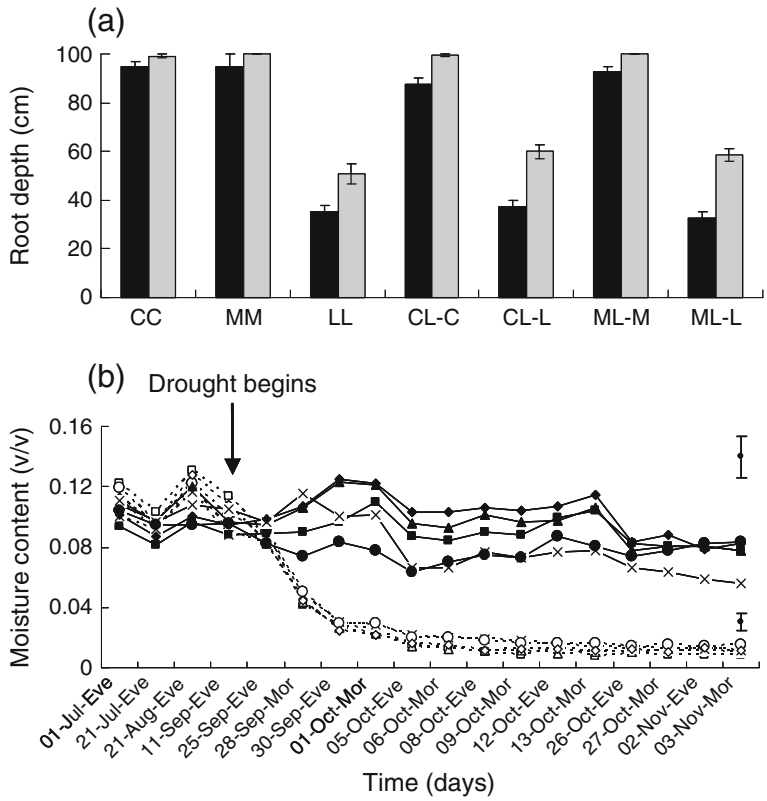

Fig. 2 **a** Root depth at the time the drought treatment began (black) and at harvest (gray) for monoculture *C. australasicum* (CC), *M. sativa* (MM), *L. rigidum* (LL) and in mixtures of *C. australasicum* grown with *L. rigidum* (CL-C), *L. rigidum* grown with *C. australasicum* (CL-L), *M. sativa* grown with *L. rigidum* (ML-M) and *L. rigidum* grown with *M. sativa* (ML-L). Note that the drought treatment had no effect at harvest (Table 1), therefore pooled estimates are given (mean±SE, n≥ 3). **b** Change in volumetric water content with time of top 30 cm soil layer of control (solid lines with filled symbols) and drought-treated (broken lines with open symbols) pots for monoculture *C. australasicum* (*squares*), *M. sativa* (*diamonds*), *L. rigidum* (*crosses*) and mixtures of *C. australasicum* and *L. rigidum* (*triangles*) and *M. sativa* and *L. rigidum* (*circles*). Bars represent 95% CI of the volumetric water content for control (*upper*) and drought (*lower*) treated pots. 'Mor' and 'Eve' along dates are for morning and evening measurements, respectively

Green leaf [N] could not be estimated for *C. australasicum* and *M. sativa* in mixtures, and drought-treated *M. sativa* in monoculture (Fig. 6a), due to insufficient sample weight. Dead leaf [N] at harvest was also affected by the species combination treatment and moisture treatment (Table 1). All the species in the drought treatment had a higher dead leaf [N] than the control plants, except for *C. australasicum* in mixtures (Fig. 6b). *L. rigidum* had a lower dead leaf [N] than *C. australasicum* and *M. sativa,* in both monocultures and mixtures. The response was quite prominent under control moisture conditions and less so in the drought treatment. Furthermore, when comparing the [N] in green and dead leaves, leaf [N] in dead leaves was lower than that of green leaves for the control moisture condition only (Fig. 6).

Concentrations of other macro- and micro-nutrient in green and dead leaves are given in Table 2. Concentrations of Ca, Na, Mg, S, Fe and Al differed among species combination treatment and between green and dead leaves, but not between moisture treatments ($F_{7,75}$>12.4, R^2>0.54). *L. rigidum* grown in both monoculture and mixtures had lower concentrations of Ca, Mg, S, Fe and Al in green and dead leaves than did *C. australasicum* and *M. sativa* in most instances. Furthermore, for all the species combination treatments the concentrations of Fe and Al in the dead leaves were higher than those of green leaves.

Concentrations of K, Co, Cu, Mo, Mn and Zn were affected by the moisture treatment and the species combination treatment, and they also differed between

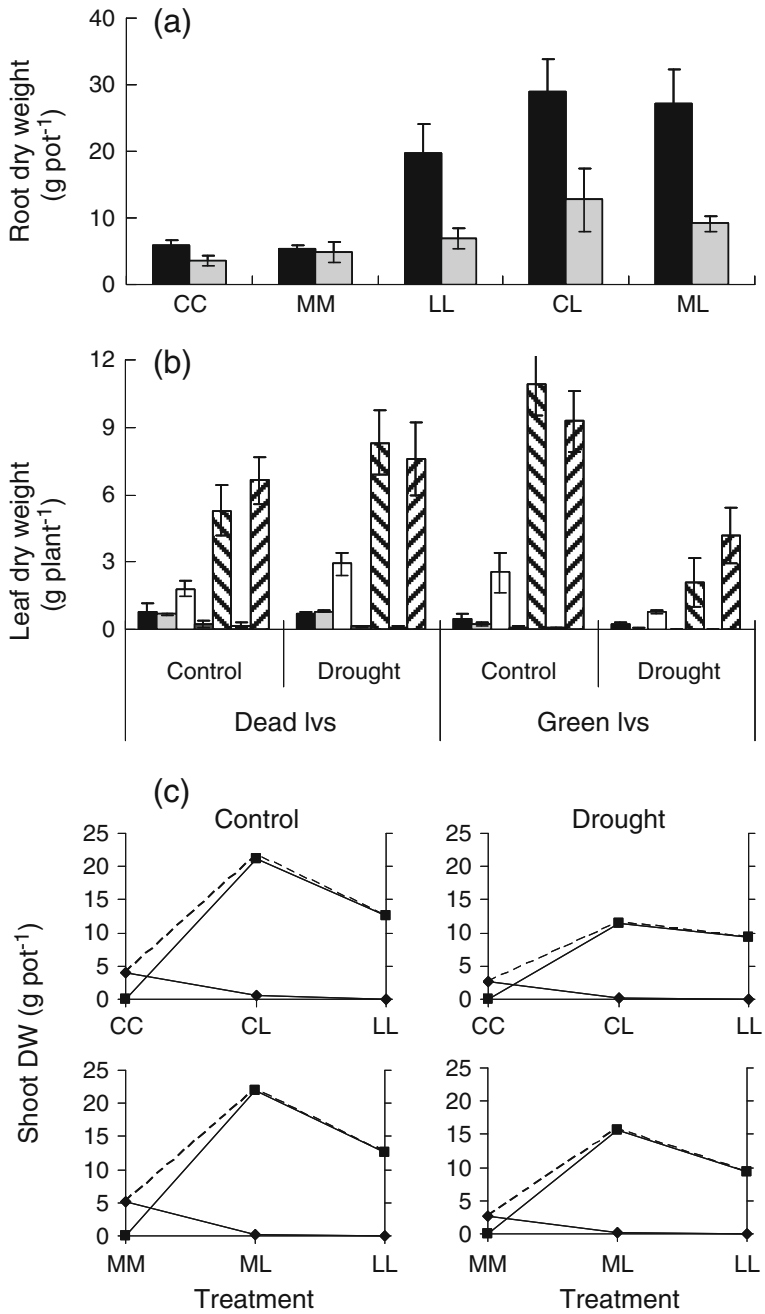

Fig. 3 **a** Root DW of monocultures of *C. australasicum* (CC), *M. sativa* (MM), *L. rigidum* (LL) and in mixtures of *C. australasicum* and *L. rigidum* (CL) and *M. sativa* and *L. rigidum* (ML) grown under control (*black*) and drought (*gray*) moisture treatments, **b** dead and green leaf DW of monoculture *C. australasicum* (*black*), *M. sativa* (*light gray*), *L. rigidum* (*white*) and in mixtures of *C. australasicum* grown with *L. rigidum* (*dark gray*), *L. rigidum* grown with *C. australasicum* (*downward hatched bars*), *M. sativa* grown with *L. rigidum* (horizontal hatched bars- almost invisible due to very low leaf DW) and *L. rigidum* grown with *M. sativa* (*upward hatched bars*) grown under drought and control moisture treatments (mean±SE, n=4), and **c** total above-ground DW of monocultures of *C. australasicum* (CC), *M. sativa* (MM), *L. rigidum* (LL), mixtures of *C. australasicum* and *L. rigidum* (CL) and *M. sativa* and *L. rigidum* (ML) under control conditions and in the drought treatment. Note: the solid line with diamonds is for either *C. australasicum* or *M. sativa*, the solid line with squares is for *L. rigidum* and the broken line is for the total aboveground DW pot^{-1}

green and dead leaves ($F_{14,68}$>7.7, R^2>0.61). When comparing moisture treatments, for green leaves of all the species combinations, Mn and Co concentrations were higher under the drought treatment than control moisture conditions. On the contrary, for both green and dead leaves, and for most of the species combination treatments, the Mo concentration was higher under control moisture conditions than under the drought treatment. When comparing species combination treatments, green and dead leaves of both *C. australasicum* and *M. sativa* had higher concentrations of Co, Cu, Mo, Mn and Zn than those of *L. rigidum*, irrespective of the moisture treatment. When comparing green and dead leaves, for all the species combination treatments, concentrations of Cu and Zn under both moisture conditions and concentration of Mo under drought

treatment, were higher in dead leaves than those in green leaves. Furthermore, due to the fact that *L. rigidum* produced higher DW and had similar or lower concentrations of all the nutrients, nutrient use-efficiency of *L. rigidum* was higher than that of *C. australasicum* and *M. sativa* (data not shown). Due to the absence of green leaves for *M. sativa* in mixtures and drought-treated *C. australasicum* in mixtures, nutrient concentrations could not be measured.

Before the drought treatment commenced, *A* was affected by the species combination treatment (Table 1), with *C. australasicum* and *M. sativa* in monoculture having higher *A* values than in mixtures (Fig. 7a). However, *A* of *L. rigidum* in monoculture was the same as that in mixtures. Three weeks after starting the drought treatment (i.e. when drought-treated *M. sativa* and *L. rigidum* were harvested), *A* was affected by the moisture treatment and the species combination treatment. All the drought-treated plants had a lower *A* than those in control moisture conditions. Drought-treated *C. australasicum* had a higher *A* than drought-treated *M. sativa* and *L. rigidum*. At final harvest (i.e. at 22 weeks), *A* was affected by moisture treatment and species combination treatment (Table 1), with *A* of drought-treated *C. australasicum* plants lower than that of control plants. Also, *A* of control plants at harvest was highly variable and values were similar to those obtained before starting the drought treatment.

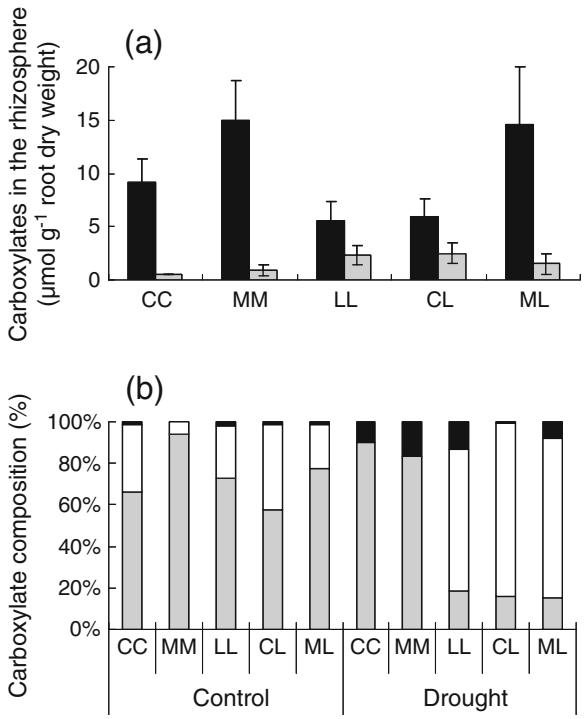

Fig. 4 **a** Concentration of carboxylates in the rhizosphere of monoculture *C. australasicum* (CC), *M. sativa* (MM) and *L. rigidum* (LL) and in mixtures of *C. australasicum* and *L. rigidum* (CL) and *M. sativa* and *L. rigidum* (ML) grown in control (*black*) and drought (*grey*) treatments (mean±SE, n≥4). **b** Percentage composition of carboxylates [citric (*grey*), malic (*white*) and others (*black*)] in rhizosphere soil collected from the top 30 cm of a pot of monoculture *C. australasicum* (CC), *M. sativa* (MM) and *L. rigidum* (LL) and in mixtures of *C. australasicum* and *L. rigidum* (CL) and *M. sativa* and *L. rigidum* (ML) grown in drought and control moisture conditions (mean of n=4). Note that the rhizosphere of the two species in mixtures couldn't be separated and therefore measurements were made for the mixtures of roots

Before the drought treatment began, g_s was affected by the species combination treatment (Table 1). *C. australasicum* and *M. sativa* in monoculture and *M. sativa* in mixtures had higher g_s values than did *L. rigidum* (Fig. 7b). *L. rigidum* plants maintained low g_s values, less than 0.6 mol m^{-2} s^{-1}, both in monoculture and in mixtures. Three weeks after the drought treatment began and at final harvest, g_s was affected by the species combination treatment and moisture treatment (Table 1). Drought-treated plants had very low g_s (Fig. 7b), while g_s of control plants at 3 weeks after the drought treatment commenced, and final harvest, were the same as that before the drought treatment began.

Water-use efficiency at harvest was affected by the moisture treatment and the species combination treatment (Table 1). At harvest, under control moisture conditions, *L. rigidum* had a higher *WUE*, both in monoculture and in mixtures (44–61 μmol mol^{-1}), than did *C. australasicum* and *M. sativa* (13–36 μmol mol^{-1}), whereas *WUE* did not differ between *C. australasicum* and *M. sativa*. However, *WUE* of drought-treated plants, 1 week before harvest, was not affected by the species combination treatment (26–67 μmol mol^{-1}).

Discussion

Growth in monocultures and mixtures

Hypothesis (i) that plants in mixtures would grow better than those in monocultures, because (a) organic anions exuded from the legume may release 'extra' P for growth by both plant species and/or (b) deep

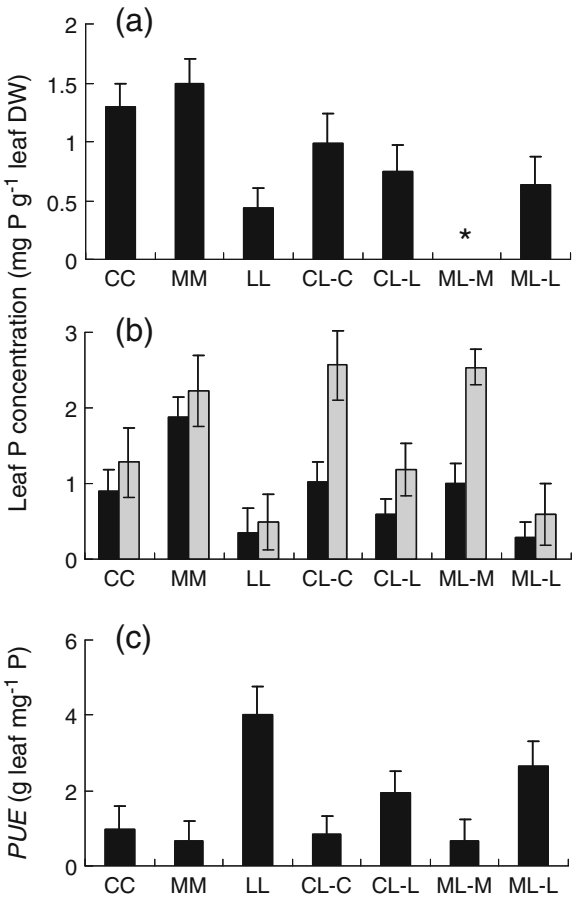

Fig. 5 P concentration in **a** green leaves and **b** dead leaves and **c** canopy phosphorus-use efficiency (*PUE*) of monocultures of *C. australasicum* (CC), *M. sativa* (MM), *L. rigidum* (LL) and in mixtures of *C. australasicum* grown with *L. rigidum* (CL-C), *L. rigidum* grown with *C. australasicum* (CL-L), *M. sativa* grown with *L. rigidum* (ML-M) and *L. rigidum* grown with *M. sativa* (ML-L). Note that green leaf [P] and *PUE* were not affected by the moisture treatment (Table 1), therefore pooled estimates are given. Dead leaf [P] was affected by the moisture treatment; control (black) and drought (gray). * indicates no green leaves were present at harvest (mean±SE, n≥4)

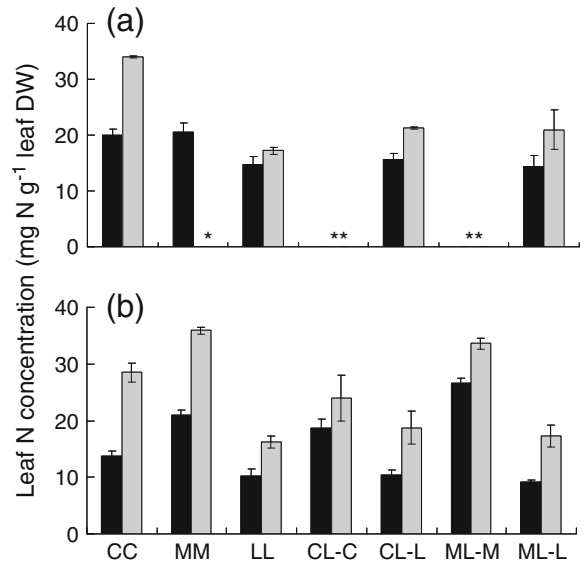

Fig. 6 N concentration in **a** green leaves and **b** dead leaves of monocultures of *C. australasicum* (CC), *M. sativa* (MM), *L. rigidum* (LL) and in mixtures of *C. australasicum* grown with *L. rigidum* (CL-C), *L. rigidum* grown with *C. australasicum* (CL-L), *M. sativa* grown with *L. rigidum* (ML-M) and *L. rigidum* grown with *M. sativa* (ML-L) grown under control (*black*) and drought (*gray*) treatments. * indicates no green leaves were found at the time of harvest (mean±SE, n≥4)

rooted legumes would lift water from depth and release it in topsoil layers to support growth by the shallow rooted grass in drying soil was only partially supported. Even though the growth of *L. rigidum* increased when it was grown with either *C. australasicum* or *M. sativa*, compared to when it was grown with a second *L. rigidum* plant, (i.e. *RYT*>1), the growth of both *C. australasicum* and *M. sativa* was more vigorous in monoculture than in mixtures. Similar results have been reported for several other species (Worster and Mundt 2007; Zhang et al. 2007) including for *L. rigidum* and *M. sativa* using bioassays (Emeterio et al. 2004). There may be several reasons for the different response of the species.

1. Allelopathic and autotoxic chemicals

Growth of *L. rigidum* in monoculture might have been negatively affected by the production of allelopathic and autotoxic chemicals, as reported by Emeterio et al. (2004) and Canals et al. (2005). Similarly the growth of both *C. australasicum* and *M. sativa* in mixtures might have been negatively affected by allelochemicals released by *L. rigidum*, as previously reported by Emeterio et al. (2004) where root elongation of *M. sativa* was greatly inhibited in the presence of shoot extracts of *L. rigidum*.

2. Rhizosphere carboxylates

Legumes (Fabaceae) may exude large amounts of carboxylates compared with grasses (Poaceae) (Pearse et al. 2006; Li et al. 2007). The literature regarding root exudates of *M. sativa* and *C. australasicum* is limited to only a few studies (Lipton et al. 1987; Masaoka et al. 1993; Gherardi and Rengel 2004; Pang et al. 2010; Suriyagoda et al. 2010a). There are no published data

on the rhizosphere exudates of *L. rigidum* or the other species when grown in mixtures. Therefore, this experiment aimed to test whether *C. australasicum* and *M. sativa* would exude more carboxylates, irrespective of the moisture treatment, and whether *C. australasicum* and *M. sativa* would facilitate access to P and enhance growth of *L. rigidum* in mixtures compared with that in monocultures (Hypothesis (i)a). The concentration of carboxylates in the rhizosphere of *C. australasicum* and *M. sativa* was highly variable and not higher than that of *L. rigidum*, except in monoculture under control moisture conditions. A growth enhancement effect on *L. rigidum* from carboxylate exudation by *C. australasicum* was further supported by the lower *PUE*, and higher aboveground DW and total P content when *L. rigidum* was grown in the mixture (i.e. 3.2 and 10.1 mg P per pot for monoculture and mixtures, respectively). The slow growth and low production of rhizosphere carboxylates by both legume species during the study might be due to the suboptimal glasshouse temperatures (Robertson et al. 2002; Suriyagoda et al. 2010b). Also, for all the species in the drought treatment, the concentration of carboxylates (μmol g^{-1} root DW) in the rhizosphere was lower than that in the control moisture condition (Fig. 4), which is not in agreement with the previous reports for monocultures of sorghum (*Sorghum bicolor* L.), *T. aestivum*, *C. australasicum* and *M. sativa* (Brady and Weil 1999; Bertin et al. 2003; Suriyagoda et al. 2010a). This disagreement might be due to microbial degradation, restricted diffusivity of the carboxylates in the rhizosphere of drought-treated

Table 2 Mean concentration of elements in the green and dead leaves of monocultures of *C. australasicum* (CC), *M. sativa* (MM), *L. rigidum* (LL) and in mixtures of *C. australasicum* grown with *L. rigidum* (CL-C), *L. rigidum* grown with *C. australasicum* (CL-L), *M. sativa* grown with *L. rigidum* (ML-M) and *L. rigidum* grown with *M. sativa* (ML-L). Estimates of ML-M under both moisture treatments and CL-C under drought treatment were not possible due to the shedding of all the green leaves. The 95% *LSD* is also given for green and dead leaf element concentrations. Concentrations of Ca, Na, Mg, S, Fe and Al did not differ between moisture treatments

Element[a]	Green leaves								Dead leaves							
	CC	MM	LL	CL-C	CL-L	ML-M	ML-L	LSD	CC	MM	LL	CL-C	CL-L	ML-M	ML-L	LSD
Ca	8.1	18.2	1.0	17.0	1.7	–	1.0	8.3	13.2	17.4	3.9	16.7	4.6	21.4	3.9	4.1
Na	16.6	5.0	3.0	0.5	5.3	–	5.5	4.0	2.5	7.1	6.8	5.0	11.7	5.5	10.7	3.9
Mg	4.9	7.8	1.1	7.8	1.7	–	1.2	1.9	5.0	8.4	3.6	7.0	4.3	7.9	3.4	2.1
S	8.8	8.3	2.5	9.2	3.8	–	2.6	3.1	10.6	11.1	6.2	13.8	7.0	10.9	5.3	3.1
Fe	220	546	98	143	122	–	86	244	707	880	195	674	237	936	146	268
Al	105	112	29	99	85	–	45	83	244	321	159	286	203	348	161	127
Control																
K	26	22	15	29	19	–	17	5	24	21	25	30	26	16	23	2.3
Co	1.1	1.4	0.5	1.1	0.6	–	0.5	0.24	1.6	2.1	0.7	2.5	0.7	3.6	0.6	0.3
Cu	17.1	31.5	11.7	18.4	12.8	–	9.9	2.7	37.0	83.4	40.4	57.9	38.6	58.5	36.8	9.4
Mo	64.2	55.2	5.9	45.6	4.5	–	2.9	5.3	70.8	64.5	18.9	35.7	11.6	16.5	5.7	6.7
Mn	689	837	167	831	218	–	167	124	891	1,041	443	1,189	419	1,184	326	95
Zn	254	325	121	239	132	–	95	24	470	557	227	411	171	478	163	47
Drought																
K	36	47	20	–	22	–	21	6	54	34	30	38	31	22	30	3
Co	2.6	5.7	0.9	–	1.6	–	1.1	1.6	3.9	4.1	1.3	3.3	1.5	2.8	1.0	0.3
Cu	18.0	21.4	14.7	–	14.4	–	14.6	3.8	42.5	37.3	27.4	51.9	26.8	63.2	27.6	5.2
Mo	6.4	2.1	2.8	–	1.6	–	1.9	1.4	43.2	22.3	8.2	21.5	3.1	27.6	4.8	3.4
Mn	1,185	1,423	256	–	410	–	283	392	1,359	1,246	442	1,247	509	1,043	375	105
Zn	278	345	172	–	197	–	188	77	501	440	255	405	232	483	191	33

[a] Ca, Na, Mg, S and K are expressed as mg g^{-1} leaf DW and Fe, Al, Co, Cu, Mo, Mn and Zn are expressed as mg kg^{-1} leaf DW

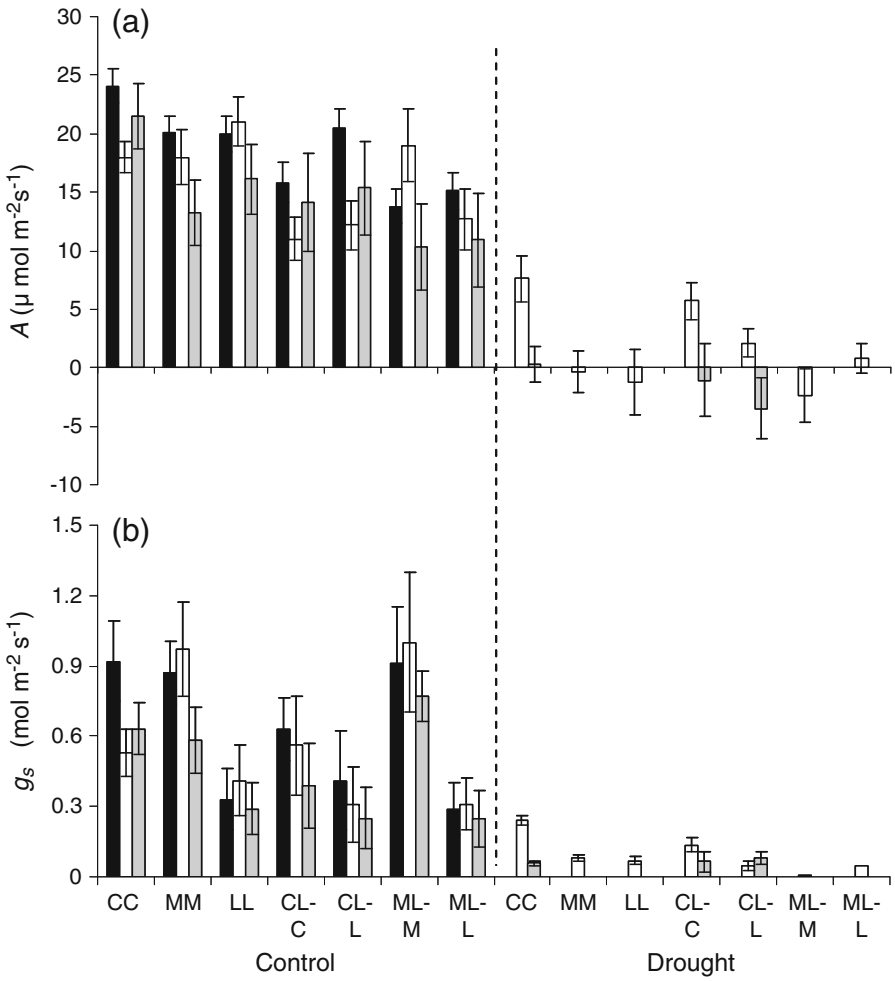

Fig. 7 **a** Photosynthetic rate and **b** stomatal conductance of monocultures of *C. australasicum* (CC), *M. sativa* (MM) and *L. rigidum* (LL) and in mixtures of *C. australasicum* grown with *L. rigidum* (CL-C), *L. rigidum* grown with *C. australasicum* (CL-L), *M. sativa* grown with *L. rigidum* (ML-M) and *L. rigidum* grown with *M. sativa* (ML-L) under control and drought treatments. Measurements were taken before the drought treatment began (*black*), 3 weeks after drought treatment (*white*) and at final harvest (*gray*). Note that A and g_s were measured 3 weeks after drought treatment began due to the early wilting/harvesting of *M. sativa* and *L. rigidum* (mean±SE, n=4)

plants and/or possible death or reduced activity of fine roots in the dry soil layers (Gerke et al. 2000).

The composition of rhizosphere carboxylates varied among species combinations and moisture treatments (a higher proportion of citric acid for all species combinations in the control and for *C. australasicum* and *M. sativa* in the drought treatment, and more malic acid for *L. rigidum* monoculture and mixtures in the drought treatment). Citrate has been reported to be the main carboxylate anion in leguminous plants (Lipton et al. 1987; Neumann and Römheld 1999; Gerke et al. 2000 and references therein) and this was the case for all the species in the control treatment in the present study. Suriyagoda et al. (2010a) reported an increase in the proportion of malic acid in the rhizosphere of severely drought-treated *M. sativa* and *C. australasicum*, in agreement with the results obtained for shallow-rooted, severely drought-stressed *L. rigidum* in the present experiment. Compared with other rhizosphere compounds, the contribution of shikimic acid was very low, except for the rhizosphere of the drought-treated *L. rigidum*. Similarly low rhizosphere shikimic acid concentrations for several other crop and tree species have been reported (Neumann and Römheld 1999; Sandnes et al. 2005). As highlighted above, the altered carboxylate composition in the drought treatment for all species might be due to differences in exudation rates, diffusivity and/or half life of carboxylates in the rhizosphere (Gerke et al. 2000; Suriyagoda et al. 2010a); it might also be associated with hydraulic redistribution, as discussed by Lambers et al. (2006). All these changes may have affected the availability of P in the dry rhizosphere. Due to the fact that hypothesis (i) a was only partly supported (i.e. growth enhancement was only present in *L. rigidum* when grown in mixtures, and a higher carboxylate concentration was found only under control moisture condition in monocultures for *M.*

sativa and *C. australasicum* than that of *L. rigidum*) further testing is warranted.

3. Hydraulic lift

The hypothesis that the deep rooted legumes would lift water from depth and release it in topsoil layers to support growth by the shallow rooted grass in drying soil (Hypothesis (i)b) was not supported. It is known that deep-rooted herbaceous legumes do exhibit hydraulic lift if their roots penetrate into deep, moist soil (Corak et al. 1987; Schenk and Jackson 2002; Sekiya and Yano 2002; Skinner et al. 2004). In particular, hydraulic lift has been observed for *M. sativa* and maize (*Zea mays* L.) intercropped under drought (Corak et al. 1987) and for several other crop and pasture species (Caldwell et al. 1998 and references there in). This discrepancy could be due to several reasons:

(a) Coarse-textured river sand might negatively influence the occurrence of hydraulic lift, perhaps due to less root-soil contact in sandy soils (less "soil suction") compared with fine-textured soils, as observed by Yoder and Nowak (1999).
(b) A rather small water potential gradient along the soil-plant continuum, due to the shedding of leaves of drought-treated plants. Also, in the present experiment, due to the fact that leaf DW and root DW of both *C. australasicum* and *M. sativa* were very low and plants were very small, irrespective of the moisture treatment, any flow of water may have been too minute for flow to be detectable.
(c) A greater senescence rate of surface-dwelling fine roots under drought conditions (Liste and White 2008), as has been shown for a savannah bunchgrass (*Aristida stricta* Minchx.) (West et al. 2003; Espeleta et al. 2004).
(d) Shallow-rooted, drought-stressed *L. rigidum* roots might have taken up the hydraulically lifted water immediately. If so, it might not have been detected in the present experiment due to measurements being taken only during late evenings and early mornings on the following day, but not continuously during the day. Even though the immediate uptake of water by the grass roots is a possibility in a legume-grass mixture, it would not occur in a legume monoculture. The fact that in the present experiment, plants in monoculture did not exhibit a change in volumetric soil moisture content, thus indicates that immediate uptake of water by *L. rigidum* roots was probably not the main reason hydraulic lift was not observed.
(e) Soil water potential is a much more sensitive measure of changes in soil water status than in soil moisture content. This is especially be true in sandy soils where very small changes in water content in drying soils would result in large changes in water potential (Yoder and Nowak 1999). Therefore, it would have been better if we could have taken soil water potential measurements using an automated continuous system throughout the experiment to detect hydraulic lift. We believe that the absence of hydraulic lift in the present experiment might be due to one or several of the reasons suggested above, and attention should be given to these when designing future experiments.

4. Use of different resources

Species with fine, extensive root systems (e.g. *L. rigidum*) have lower external [P] requirements for maximum growth than species with thick, small root systems (e.g. *M. sativa* and *C. australasicum*) (Hill et al. 2006). The sparingly soluble $FePO_4$ might have produced adequate available P for *L. rigidum* to maintain its maximum growth and the observed differences in growth might be due to the early superior growth response of *L. rigidum* to low external [P].

Nutrient dynamics

Hypothesis (ii) was that nutrients would be remobilised to green leaves from senescing leaves and that the response would be more prominent under control moisture conditions. This hypothesis was supported only for *C. australasicum* and *L. rigidum* in control moisture conditions, where dead leaf [N] was reduced compared with green leaf [N] (N-resorption); such a response was not observed for other nutrients. The absence of such a response might be due to the abundant availability of nutrients provided by the large soil volume amended with favourable concentrations of nutrients. High concentrations of Al, Mn, Co, Cu, Zn and Mo in the green and dead leaves of both *C. australasicum* and *M. sativa,* compared with those in *L. rigidum*, irrespective of the moisture treatment, might be due to the legumes' slower growth rate and/or to higher uptake rates made possible by acidification of the rhizosphere by exuded

carboxylates. In agreement with our results, similarly high Mn concentrations in leaves of *M. sativa* have also been reported elsewhere (Hayes et al. 2008). On the other hand, the higher nutrient-use efficiency (e.g. *PUE*) (Fig. 5) of *L. rigidum* compared to that of *C. australasicum* and *M. sativa* might be due to a "dilution effect" resulting from the higher DW produced. These results highlight the importance of investigating these species for their potential to be used in phytoremediation and the quality of the pasture produced under the availability of low-P and drought conditions.

Growth of *C. australasicum* and *M. sativa*

M. sativa has long been grown in fertile and moist environments in Asia (Griffiths 1949; Small 2009) and is not well adapted to areas with low summer rainfall (Cocks 2001; Dear et al. 2007). Conversely, many native Australian perennial legumes have evolved in dry P-impoverished landscapes (Beadle 1966; Handreck 1997) and are expected to resist drought better than *M. sativa*. However, Hypothesis (iii) that *C. australasicum* would perform better (i.e. greater P uptake, higher *A* and tighter stomatal control and faster growth) under drought in both monoculture and mixtures when compared with *M. sativa* was only partially supported. Total above-ground DW, and green and dead leaf nutrient concentrations of *C. australasicum* in monoculture and mixtures were similar to those of *M. sativa*, irrespective of the moisture treatment. Recently, in a P-response study using KH_2PO_4, Pang et al. (2010) reported that both *M. sativa* and *C. australasicum* had a similar growth response, and achieved maximum growth (DW) at 24 mg P kg^{-1} dry soil. Our results on DW are in agreement with this. However, *M. sativa* plants exposed to the drought treatment died 3 weeks after the treatment began, whereas *C. australasicum* plants shed most of their mature leaves and remained alive with only a few apical leaves intact, which supports our hypothesis. Furthermore, drought treated *C. australasicum* plants maintained higher *A* and g_s values than *M. sativa* did and thus survived drought much longer. A similar more plastic growth response of *C. australasicum* in comparison with *M. sativa* has also been reported by Suriyagoda et al. (2010a).

Both *M. sativa* and *C. australasicum* had poor growth compared with *L. rigidum* in the presence of $FePO_4$ and in mixtures. Thus, to attain a good establishment for both the legumes under field conditions, a wider time gap between the planting of legume and the grass, as well as an adequate available-P pool in the soil, is important.

Concluding remarks

The growth of *C. australasicum* and *M. sativa* in mixtures was decreased while the growth of *L. rigidum* was increased compared with that in monocultures resulting in a $RYT>1$ in mixtures. The extensive root system of *L. rigidum* favoured the uptake of nutrients and moisture to support its growth and, in addition, *L. rigidum* may have benefited from rhizosphere P solubilisation due to carboxylates released by the legume companion. Also, *L. rigidum* maintained tighter stomatal control than *M. sativa* and *C. australasicum*, thus conserving the limited soil moisture available for its shallow root system. We found no evidence for our hypothesis of hydraulic lift. Both *C. australasicum* and *M. sativa* had higher concentrations of most of the macro- and micro-nutrients in their leaves compared with *L. rigidum*. *C. australasicum* did not produce more DW or show a more efficient nutrient utilisation than *M. sativa*, but it survived longer in the drought treatment than did *M. sativa*.

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