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Biomass production and water use efficiency in perennial grasses during and after drought stress

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Abstract

Drought is a great challenge to agricultural production, and cultivation of drought-tolerant or water use-efficient cultivars is important to ensure high biomass yields for bio-refining and bioenergy. Here, we evaluated drought tolerance of four C_3 species, *Dactylis glomerata* cvs. Sevenop and Amba, *Festuca arundinacea* cvs. Jordane and Kora, *Phalaris arundinacea* cvs. Bamse and Chieftain and *Festulolium pabulare* cv. Hykor, and two C_4 species *Miscanthus × giganteus* and *M. lutarioriparius.* Control (irrigated) and drought-treated plants were grown on coarse and loamy sand in 1 m^2 lysimeter plots where rain was excluded. Drought periods started after harvest and lasted until 80% of available soil water had been used. Drought caused a decrease in dry matter yield (DM; $P < 0.001$) for all species and cultivars during the drought period. Cultivars Sevenop, Kora and Jordane produced DM at equal levels and higher than the other C_3 cultivars in control and drought-treated plots both during and after the drought period. Negative correlations were observed between stomatal conductance (g_s) and leaf water potential ($P < 0.01$) and positive correlations between g_s and DM ($P < 0.05$) indicating that g_s might be suitable for assessment of drought stress. There were indications of positive associations between plants carbon isotope composition and water use efficiency (WUE) as well as DM under well-watered conditions. Compared to control, drought-treated plots showed increased growth in the period after drought stress. Thus, the drought events did not affect total biomass production (DM_{total}) of the whole growing season. During drought stress and the whole growing season, WUE was higher in drought-treated compared to control plots, so it seems possible to save water without loss of biomass. Across soil types, *M. lutarioriparius* had the highest DM_{total} (15.0 t ha^{-1}), WUE_{total} (3.6 g L^{-1}) and radiation use efficiency (2.3 g MJ^{-1}) of the evaluated grasses.

Keywords: bioenergy, bio-refining, carbon isotopic composition, drought tolerance, dry matter yield, intercepted photosynthetically active radiation, leaf water potential, radiation use efficiency, ratio vegetation index, stomatal conductance

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Introduction

Drought can be defined as a soil moisture deficit level causing social, environmental and/or economic impacts (Emerson *et al.*, 2014), and it is considered to be a major environmental constraint to plant productivity (Farooq *et al.*, 2009) and thereby agricultural production (Boyer, 1982). Droughts are expected to increase in intensity and/or duration with climate change, as these changes are predicted to entail higher global mean surface temperatures (IPCC, 2013) or increased aridity in many regions of the world (FAO, 2011). This might put an even higher pressure on freshwater reserves, as 20% of the world's cultivated land is irrigated contributing to about 40% of the crop production (Bruinsma, 2003) and worldwide agriculture accounts for approx. 70% of the freshwater withdrawals (FAO, 2011). However, renewable freshwater accessible for human use is a limited resource (Postel *et al.*, 1996). Furthermore, increased demands for food and bio-based materials and energy from a growing human population require intensified biomass production. Consequently, a more sustainable use of water in agriculture is necessary (Morison *et al.*, 2008), such as cultivation of crops with a high tolerance of water deficit or drought (Pfister *et al.*, 2011). This would also be useful if the land area with potential for crop production is expanded, as some regions might be impossible to irrigate due to high irrigation charges or geographical location far from water supplies. These

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areas may be classified as marginal land not suitable for production of traditional food and feed crops, but rather for alternative drought-tolerant crops suitable as feedstock for biofuels and bioenergy (Tilman *et al.*, 2009) or for bio-refining into both food, energy and materials (Parajuli *et al.*, 2015).

Drought tolerance is the ability of plants to maintain metabolism and biomass production at low water potentials caused by limited water availability (Jones *et al.*, 2015), which also results in higher water use efficiency (WUE). Cultivation of plant genotypes with high drought tolerance or WUE can contribute to yield increases in agriculture (Tilman *et al.*, 2002). Perennial rhizomatous grasses are interesting to use as biomass crops (Lewandowski *et al.*, 2003) because they often have high drought tolerance in addition to high yield potential, high lignin and cellulose content and low negative environmental impacts (Jorgensen, 2011; Smith *et al.*, 2013). Although drought may reduce also the quality of biomass feedstocks (Emerson *et al.*, 2014), yield has been identified as the most important factor in biomass production for biofuel generation (Styles *et al.*, 2008).

Plants exhibit a range of characteristics and mechanisms to cope with limited water availability including many physiological, morphological and molecular responses (Farooq *et al.*, 2009; Jaleel *et al.*, 2009). For example, it has been found that drought tolerance in cocksfoot was associated with dense tiller formation and reduced leaf production (Harris *et al.*, 2008) and a deep root system that makes it possible to exploit larger soil volumes (Volaire *et al.*, 1998b). Furthermore, drought tolerance is proposed to be improved by changes in photosynthetic pigments (Jaleel *et al.*, 2009).

In most plants, lack of water initiates stomatal closure and common indicators of drought stress and tolerance are stomatal conductance (g_s; Chaves *et al.*, 2002) and leaf water potential (Ψ_l; Matin *et al.*, 1989). It would be an advantage if drought-tolerant or water use-efficient genotypes could be selected using such alternative faster and more effective methods than measurement of dry matter yield (DM), which is performed at the end of the growing season and therefore is time-consuming (and expensive). Carbon isotope discrimination ($\Delta^{13}C$) may also be used as an indirect measure of WUE in C_3 species (Cattivelli *et al.*, 2008), but has so far not been found applicable to plants with C_4 photosynthesis (Chen *et al.*, 2011). The $\Delta^{13}C$ is calculated based on the plants carbon isotope composition ($\delta^{13}C$). Both $\Delta^{13}C$ and $\delta^{13}C$ have been used in an attempt to estimate WUE, and due to the different definitions (Chen *et al.*, 2011), they show different relations to WUE, that is usually negative ($\Delta^{13}C$) or positive ($\delta^{13}C$) correlations, respectively.

The aim of this study was to determine the effect of drought stress on above-ground productivity of different species and cultivars of perennial grasses and moreover to evaluate potential indicators of drought tolerance and WUE. Furthermore, we examined whether the drought stress affected the regrowth of the grasses in the fully irrigated period after drought treatments.

Materials and methods

Plant material

Six perennial grass species were investigated (Table 1). These included three species, with two cultivars of each, grown in a completely randomized block design: *Dactylis glomerata* L. (cocksfoot) cvs. Sevenop and Amba, *Festuca arundinacea* Schreb. (tall fescue) cvs. Jordane and Kora and *Phalaris arundinacea* L. (reed canary grass) cvs. Bamse and Chieftain. In addition to two species of miscanthus, *Miscanthus × giganteus* J.M.Greef & Deuter ex Hodk. & Renvoize 'Hornum' and *M. lutarioriparius* L.Liu ex S.L.Chen & Renvoize, and *Festulolium pabulare* (festulolium) cv. Hykor, which is a hybrid between *Lolium multiflorum* L. × *F. arundinacea* L.

Growth conditions

Semifield experiments were carried out at AU-Foulum (56°30′ N, 9°34′ E) on two types of soil collected at two experimental stations: (i) at Jyndevad, coarse sand (Danish soil classification JB 1), and (ii) at Foulum, loamy sand (JB 4), respectively. In 1992, the drainable concrete lysimeters were filled with soil. Each lysimeter has a length and width of 1.0 m by 1.0 m and a depth of 1.4 m. Further information of the construction (Sørensen & Rubæk, 2012) and physical properties of the soil types can be found in Table 2 and Ahmadi *et al.* (2010). A mobile roof automatically covered the lysimeters in case of rain. A total of 108 lysimeters were placed in six rows, where 54 lysimeters contained one soil type and 54 lysimeters were filled with the other soil type (Fig. S1). Two rows for each soil type constituted one replicate. Each replicate comprised 18 lysimeter plots including two plots for each of the seven grass cultivars and two miscanthus species. One plot was for the control and the other one for the drought-treated plants. Thus, the six cultivars of cocksfoot, tall fescue and reed canary grass were grown in a completely randomized block design with three replicates for each soil type. Miscanthus species were placed in the north-west (N-W) end of each of the two randomized blocks to avoid shadow effects on the other shorter cultivars. Hykor was located right N-W of the miscanthus and acted as border plants. However, during the experimental period Hykor was not affected by shadows from the taller miscanthus, and results from Hykor are therefore included without statistical assessments.

In 2014, the precrop (2013) was barley and the lysimeter plots were prepared for the experiments by digging the soil between 24 and 28 March. The grass cultivars and miscanthus

Table 1 Species and cultivars included in the experiment with indication of photosynthetic type and the measurements performed

Species	Cultivar	Photosynthesis	Measurements	
			2014	2015
Dactylis glomerata	Amba	C_3	All*	All†
	Sevenop	C_3	All*	All†
Festuca arundinacea	Kora	C_3	All*	All†
	Jordane	C_3	All*	All†
Phalaris arundinacea	Bamse	C_3		All†
	Chieftain	C_3		All†
Miscanthus × giganteus		C_4		RVI, Ψ_l, g_s, DM_{total}, WUE_{total}, RUE_{total}, $IPAR_{total}$
M. lutarioriparius		C_4		RVI, Ψ_l, g_s, DM_{total}, WUE_{total}, RUE_{total}, $IPAR_{total}$
Festulolium pabulare	Hykor	C_3		All†

*Dry matter yield (DM), water use efficiency (WUE), ratio vegetation index (RVI), leaf water potential (Ψ_l) and stomatal conductance (g_s).

†DM, DM reduction (DM_{red}), WUE, carbon isotopic composition ($\delta^{13}C$), RVI, Ψ_l, g_s, DM after the drought period (DM_{adp}), total DM (DM_{total}), total WUE (WUE_{total}), total radiation use efficiency (RUE_{total}) and cumulative intercepted photosynthetically active radiation ($IPAR_{total}$).

Table 2 Physical properties and available water capacity of the soils in the lysimeters

Soil	Texture	Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	Bulk density (g cm^{-3})	Available water capacity (Vol%)
Jyndevad	Coarse sand	0–30	5.8	2.1	90.2	1.9	1.40	10.2
	Coarse sand	30–70	5.9	0.5	92.9	0.7	1.53	6.0
Foulum	Loamy sand	0–30	11.5	11.0	75.2	2.3	.29	18.6
	Loamy sand	30–70	14.9	10.1	74.5	0.5	1.56	13.7

species were established during spring 2014. Seeds of all grasses except miscanthus were broadcast on 8 April 2014 with 45 kg ha^{-1} for cvs. Sevenop, Amba, Jordane and Kora and 50 kg ha^{-1} for cvs. Bamse, Chieftain and Hykor, which gives seeding rates about twice as high as standard. After evaluation of shoot emergence, seeds were added for cvs. Bamse, Chieftain and Hykor (30, 30 and 40 kg ha^{-1}, respectively) on 3 June and covered with one cm of soil to obtain dense and uniform crops. This was also done for cvs. Sevenop, Amba, Jordane and Kora on 17 June (40 kg ha^{-1}). Seeds of Chieftain were added again and sown in rows on 8 July (40 kg ha^{-1}).

Plugs of the two species of miscanthus were planted by hand on 28 May 2014 with 13 plants m^{-2}. They were irrigated immediately after planting to ensure good contact between the roots and the soil. Plant survival was evaluated during summer, and replacement of dead plants was done on 1 October. All miscanthus plants were supplied by Tinplant GmbH, Klein-Wanzleben, Germany, where they had been micropropagated from mother plants, transferred to soil in 2013 and grown in small pots in an unheated greenhouse during the winter. The mother plants of *M. lutarioriparius* and *M. × giganteus* were provided by Kai-Uwe Schwarz, JKI Braunschweig, Germany, and Susanne Barth, Teagasc, Ireland, respectively.

All grasses, except miscanthus, were cut by hand at a height of approx. 10 cm above the soil surface between 17 and 22 July and the biomass removed from the plots. This was repeated for the cvs. Sevenop, Amba, Jordane, Kora and Hykor on 8 August right before the 2014 drought treatment started. Cultivars Bamse and Chieftain were cut on 15 September, cv. Hykor on 8 October and all grasses except reed canary grass and miscanthus were cut on 4 November. In 2015, miscanthus was cut to 10 cm on 12 March. Harvests for measurements are described below.

Fertilization, weeding and fungicide treatment

In 2014, 125 kg N ha^{-1} (in NPK with 16% N) was added to all plots on 19 May, except miscanthus. In 2015, 300 kg N ha^{-1} (NPK 18-4-14) divided into three portions of 100 kg N ha^{-1} was supplied on 8 April, 15 May and 27 July, respectively, to all plots except miscanthus. Miscanthus was fertilized with 100 kg N ha^{-1} (NPK 18-4-14) on 15 May. In 2014, all plots were weeded by hand on 4, 10 or 11 June and thereafter when necessary to keep them free from weeds. In 2015, all plots were weeded by hand on 8 April and 28 May and when necessary, and all plots were treated against mildew (*Erysiphaceae*) with Amistar (0.5 l ha^{-1}) on 28 May and 22 June.

Soil water content, irrigation and drought treatment periods

Volumetric water content of the soil in replicate 1 of each treatment was determined by two-rod time domain reflectometry (TDR) sensors (Plauborg *et al.*, 2005). Vertically installed probes with 80-cm-long rods and horizontally installed probes at

100 cm depth with 50-cm-long rods were used. Field capacity (FC) is defined as soil water content (% by volume) two to three days after it has been wetted thoroughly by rain (or irrigation) that has subsequently drained off. The FC was measured on 20 and 27 May 2014. The root zone available water capacity (RZAWC, Table 2) was estimated to be 61 and 125 mm water for coarse and loamy sand, respectively, based on laboratory measurements of soil water retention and assuming an effective rooting depth of 80 cm. The imposed drought period was ended when 80% of RZAWC was used, which corresponds to 46 and 100 mm water on coarse and loamy sand, respectively.

In 2014, the development of soil water content was monitored by TDR measurements twice a week from 8 August until 13 October, which are the dates of the initiation and termination of the drought stress treatment. However, due to lower water-holding capacity of the coarse sandy soil compared to the loamy sand, the drought treatment on coarse sand was terminated on 19 September by irrigation. In 2015, the drought period was from 21 May until 2 July. On 10 June, some of the plots on coarse sand had reached the drought level of 80% deficit and all coarse sandy plots were irrigated to field capacity (Fig. 1b, Table 3). Thereafter the drought treatment was reiterated lasting until 2 July when the 80% drought level was reached in some plots on both soil types (Fig. 1a,b). Development of soil water content in 100 cm depth was monitored by TDR measurements from 8 June until 2 July 2015 (Fig. 1a,b). The control plots were well-watered throughout the seasons in 2014 and 2015, as were the drought-treated plots outside the drought treatment periods. This was ensured by irrigation to FC (Table 3) at 20–25 mm deficit. Based on TDR measurements and water-deficit calculations for replicate 1, all three replicates of each treatment were irrigated with the same amount of water by a programmable irrigation machine delivering water individually to each plot at low irrigation intensity.

Plant measurements

Physiological measurements were performed on plants growing within the centre of each plot avoiding the outermost 20 cm at each side to prevent deviations in growth induced by possible border effects.

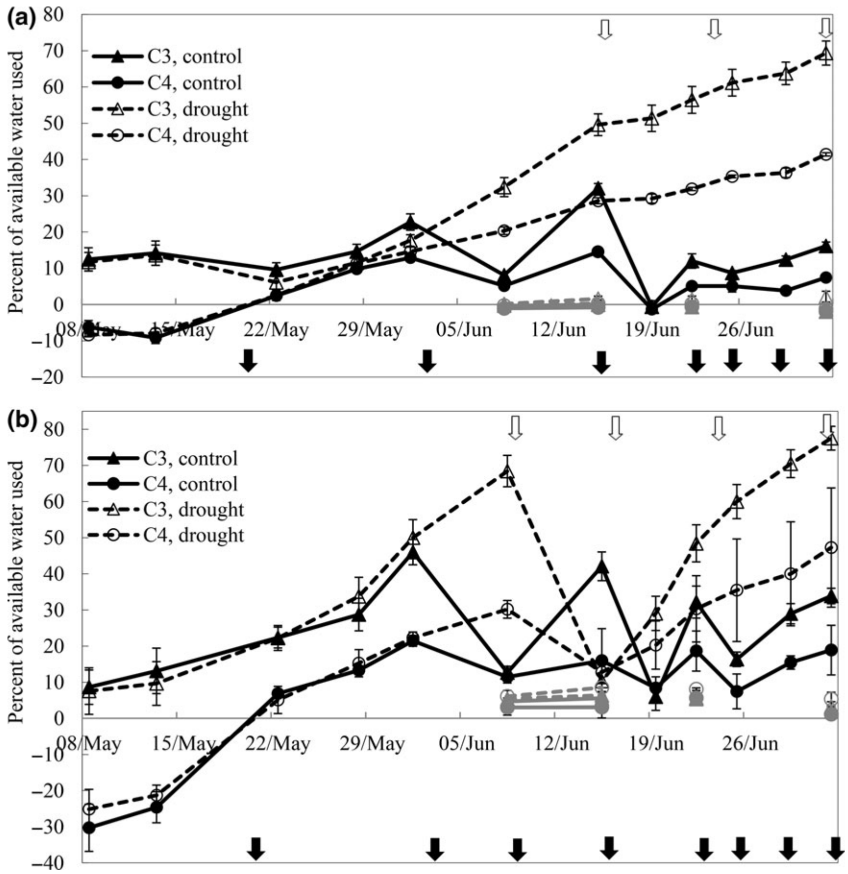

Fig. 1 Soil water deficit illustrated as per cent of available water used in the control and drought-treated plots as an average of the seven C_3 and two C_4 grasses on (a) loamy and (b) coarse sand. Measurements are vertically in 0–80 cm depth (black symbols) from 8 May until 2 July 2015 and horizontally in 100 cm depth (grey symbols) from 8 June until 2 July. The drought level, which was 80% of the available soil water used, corresponded to 100 and 46 mm on loamy and coarse sand, respectively. The arrows indicate timing of stomatal conductance and leaf water potential measurements (open) and timing of irrigation (filled). Error bars represent SE (n = 7 for C_3 and n = 2 for C_4).

In 2014, measurements were taken on cocksfoot cvs. Amba and Sevenop and tall fescue cvs. Kora and Jordane (Table 1). The results from this experiment were only preliminary as it was performed in the year of establishment. In 2015, measurements were taken in all nine grasses (Table 1). However, as miscanthus plants are only harvested once a year at the end of the growing season, they could not be measured for DM, WUE and δ^{13}C during the drought treatment period.

Stomatal conductance and leaf water potential

In 2014, stomatal conductance (g_s) was measured with a leaf porometer (Model SC-1; Decagon, Pullman, WA, USA) on the abaxial surface of fully developed and sunlit leaves. In 2015, only the two miscanthus species were measured on the abaxial side, whereas all other cultivars were measured for g_s on the adaxial side. This was done because miscanthus was observed to have higher g_s on the abaxial than the adaxial surface, whereas the other grasses had the highest g_s on the adaxial side. Due to time constraints, it was not possible to perform the measurements on both sides of the leaves.

For each cultivar and replicate, g_s and leaf water potential (Ψ_l) were measured on control and drought-treated plants immediately after each other. Measurements were conducted on surface-dry leaves between 10:00 and 14:00 h in full sunlight on both soil types 20, 27, 35 and 41 days after the drought treatment had started in 2014. One additional measurement was made for loamy sand 61 days after initiation of the progressive soil drying because the water-holding capacity was higher in this soil type and it therefore took longer before the drought level was reached. The timing of these measurements in 2015 is shown in Fig. 1a,b. Immediately after each leaf had been measured for g_s, it was wrapped in a polyethylene bag and instantaneously detached with a sharp scalpel ten cm above the soil surface. The bag was wrapped around the leaf leaving two cm of the cut end visible outside the bag. It was fixed with a paper clip, put in a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA) and measured for Ψ_l. This was done by gradually increasing the pressure and observing the cut end of the petiole through a binocular microscope. When the xylem water became visible, the balancing pressure was read.

Canopy spectral reflectance

The canopy spectral reflectance was measured using a handheld RapidSCAN CS-45 sensor (Holland Scientific, Lincoln, NE, USA). For each of the plots, the sensor was held centrally 50 cm from the side borders, 30 cm from one of the other borders and 30 cm above the crop. Then, the measurement was started as the sensor was moved horizontally 40 cm towards the border opposite to this. Red (R) and near infrared (NIR) light reflectance at 670 and 780 nm, respectively, were measured and the ratio vegetation index (RVI) was calculated as: RVI = NIR/R. The daily fraction of intercepted photosynthetically active radiation (f_{ipar}) and cumulative intercepted photosynthetically active radiation (IPAR) was calculated as described in Vargas *et al.*, 2002 using daily values of global radiation (Q) from the local weather station (56°29′ N, 9°34′ E) and assuming that

$$\mathrm{PAR} = 0.5 \times Q\,(\mathrm{MJ\,day^{-1}\,m^{-2}}) \quad (1)$$

In 2014, the canopy spectral reflectance was measured for both soil types once a week from 1 September until 4

Table 3 Irrigation during (19 May–2 July) and after (3 July–7 October) the drought stress period in the control and drought-treated plots of nine perennial grasses on coarse and loamy sand in 2015. The drought-treated plots on the coarse sandy soil were irrigated to field capacity 10 June as they reached the drought level faster than plots on loamy sand. After the drought period, all plots were fully irrigated at 20–25 mm deficit. Precipitation before and after the drought period is indicated

	Irrigation (mm)								Precipitation (mm) All plots§ Both soil types
	Control plots				Drought-stressed plots				
	Drought period*		After drought†		Drought period*		After drought†		
Cultivar	Coarse	Loamy	Coarse	Loamy	Coarse‡	Loamy	Coarse	Loamy	
Amba	105	130	20	25	40	0	40	100	142 + 201
Sevenop	130	115	15	15	35	0	50	90	142 + 201
Kora	135	130	20	25	55	0	55	95	142 + 201
Jordane	100	105	20	20	35	0	50	80	142 + 201
Bamse	105	95	25	15	35	0	40	65	142 + 201
Chieftain	130	105	20	25	45	0	45	80	142 + 201
M. × *giganteus*	50	60	10	10	20	0	20	50	142 + 201
M. lutarioriparius	75	60	15	10	20	0	40	50	142 + 201
Hykor	105	135	25	20	50	0	50	90	142 + 201

*21 May to 1 July.

†2 July.

‡One irrigation event 10 June.

§Precipitation: 1 March to 19 May + 3 July to 7 October.

November, except 2 October. In 2015, it was measured once a week from 8 April until 6 October, except 15 and 22 April and 9 September. Daily values of RVI were estimated by linear interpolation between measurement dates.

Biomass yield, radiation and water use efficiency

In both years, the grasses were cut at a height of approx. 10 cm above the soil surface. For coarse and loamy sand, the harvest was carried out on 19 September and 13 October 2014, respectively, when the drought treatment periods ended. In 2015, all grasses except miscanthus were harvested on 19 May, just before initiation of the drought treatment period, 2 July, at the end of drought treatment periods, and 7 October to determine regrowth of grasses under fully irrigated and rainfed conditions after the drought treatment periods. The drought-treated plants on coarse sandy soil that were irrigated on 10 June were harvested on 2 July at the end of the second drought period.

Miscanthus was harvested on 7 October only. All the above-ground biomass from each plot was collected in a plastic sack and weighed. The plant material was dried at 60 °C for 48 h, whereafter it was weighed and the DM was determined. The dry matter yield reduction (DM_{red}) was calculated for each replicate separately as the difference between DM in control and drought-treated plots of each grass species during the drought stress period:

$$DM_{red} = DM_{control} - DM_{drought} \quad (2)$$

In 2015, the DM of the third harvest of cvs. Amba, Sevenop, Kora, Jordane, Bamse, Chieftain and Hykor was designated the dry matter yield production in the period after the drought treatment (DM_{adp}). The plots were termed drought treated and control, even though no plots were deficient of water in the postdrought period until harvest on 7 October. The total dry matter yield (DM_{total}) was calculated by adding the DM from the three harvests for each of these seven cultivars. The DM_{total} of *M. lutarioriparius* and *M. × giganteus* was equal to DM harvested on 7 October.

The radiation use efficiency (RUE) was calculated from DM and IPAR of each plot for the respective periods as:

$$RUE = \frac{DM}{IPAR} \quad (3)$$

The water use efficiency for above-ground biomass in 2015 was calculated as DM produced divided by water supplied through irrigation and precipitation (Table 3) during the drought stress period and for the whole growing season from 1 March until harvest 7 October. The precipitation data were collected from the local weather station.

Carbon stable isotope composition analysis

All samples that were harvested and dried at the end of the drought treatment 2 July 2015 (C_3 grasses) were milled on a Foss Cyclotec 1093 (based on Tecator™ technology). After carefully mixing each sample separately, a portion of approx. two times 5 ml was randomly drawn from each sample and ground for two minutes on a Retsch MM 400 ball mill (Retsch Gmbh, Haan, Germany). This resulted in a fine powder, which was dried at 60 °C overnight. From each of these samples, between 2 and 3 mg was weighed into a small tin capsule for solid samples (Costech international S.p.A). These were analysed for carbon isotopic composition expressed by delta notation ($\delta^{13}C$) defined as parts per thousand ($^o/_{oo}$) deviating from the standard material Pee Dee belemnite (PDB), calculated according to:

$$\delta^{13}C\,(‰) = ((R_{sample}/R_{standard}) - 1) \times 1000 \quad (4)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio in the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the standard.

The carbon isotopic ratio analysis was performed at University of Copenhagen, Department of Geosciences and Natural Resource Management. The samples were analysed using Dumas combustion on an elemental analyser (EA1110, Thermo Scientific, Milan, Italy) coupled in continuous flow mode to an isotope ratio mass spectrometer (Delta Plus, Thermo Scientific, Bremen, Germany; P. Ambus, personal communications).

Statistics

As described above the experiment was a completely randomized block design for each soil type, except that the two miscanthus species always were located in the N-W end of each soil type. Therefore, the two soil types could not be compared statistically. Similarly, the two miscanthus species could not be compared using a simple linear model. The festulolium cv. Hykor was not included in the statistical analysis as it acted as border.

To examine the effect of different factors and their jointly variation, a multivariate multifactorial linear model was applied. When comparing the two miscanthus species with the six C_3 cultivars, we made an approximate analysis by considering the design as an incomplete block design. Here, we regarded each pair of columns within each replicate and soil type as an incomplete block. The model included the variation between replicates together with the main effect and two-way interactions between the factors Cultivar, Drought treatment and Soil type. The different levels of each factor (except soil type) and combination of two factors were tested using an *F*-test. If the *F*-test was significant ($P < 0.05$), pairwise comparisons were performed using *t*-tests (except for levels of soil type). The joint variation of the main effect of cultivars and the two-way interaction were measured by Pearson's coefficient of correlation (between means of the effect) while the random joint variation was measured by Pearson's coefficient of correlation between the residuals. Calculations were performed using the procedures GLM and mixed of SAS (SAS Institute Inc., 2011).

Results

Dry matter yield during drought stress

During the drought stress period from 21 May until 2 July 2015, all cultivars individually (across soil types) had lower DM in drought-treated plots than in irrigated

($P < 0.001$; Fig. 2). Also the average DM of drought-stressed treatments across soil types (2.59 t ha^{-1}) was significantly lower ($P < 0.001$, Table S1) than the average of the control (3.68 t ha^{-1}). Across treatments and soil types, the highest DM of 3.64 t ha^{-1} was measured in cv. Sevenop and the lowest 2.36 t ha^{-1} DM in cv. Bamse ($P < 0.001$). The average DM of cvs. Sevenop, Kora and Jordane (3.64, 3.55 and 3.48 t ha^{-1} DM, respectively) were all significantly higher than of cv. Amba (3.03 t ha^{-1} DM; $P < 0.001$). Furthermore, the average yields of these four grasses were significantly higher than the average yields of cvs. Bamse and Chieftain (2.36 and 2.75 t ha^{-1} DM, respectively, $P < 0.001$). Cultivar Hykor had a mean DM of 3.53 t ha^{-1} across treatments and soil types. Cultivar Hykor was not randomized with the other grasses, but still this indicated that the yield for this cultivar was at least at a similar level as cvs. Sevenop, Kora and Jordane.

As observed in 2015, also during the drought stress period in 2014 cv. Sevenop had the highest yield (3.08 t ha^{-1} DM) across treatments and soil types ($P < 0.05$, data not shown). Similarly to 2015, in 2014 the DM was lower in the drought-treated compared to the control plots for all individual cultivars across soil types ($P < 0.001$). Furthermore in 2014, the average DM of the four grass cultivars was significantly higher in control (3.30 t ha^{-1}) compared to drought-treated (2.39 t ha^{-1}) plants (across soil types; $P < 0.001$). Although not tested statistically, the average DM in 2014 on loamy sand (3.38 t ha^{-1}) was larger than on coarse sand (2.32 t ha^{-1}).

Dry matter yield reduction

For all cultivars and on both soil types, there was a reduction in DM as a result of drought stress, but significant DM_{red} differences between cultivars were only found on loamy sand (Fig. 3). The largest yield difference between control and drought-treated plots was found in Sevenop (1.51 t ha^{-1}) and the smallest in Jordane and Kora (0.70 and 0.73 t ha^{-1}, respectively) on loamy sand. The DM_{red} of Jordane and Kora was significantly smaller than of Sevenop ($P < 0.01$) and Bamse ($P < 0.05$). The DM_{red} in Chieftain and Amba was also significantly smaller than in Sevenop ($P < 0.05$).

Dry matter yield after the drought period

For all cultivars, except cv. Jordane, the DM production in the period after the drought treatment (DM_{adp}) was higher in the plots that had previously been drought-stressed compared to the control plots (Fig. 2). The difference was significant for cvs. Sevenop and Chieftain ($P < 0.05$). As for Jordane, it seemed that cv. Hykor had similar DM_{adp} in the control and previously drought-treated plots (Fig. 2). Across soil types and cultivars, the average DM_{adp} of the drought-treated plots (5.19 t ha^{-1}) was higher than the control plots (4.71 t ha^{-1}; $P < 0.01$).

The average DM_{adp} of the cultivars across treatments and soil types was significantly different ($P < 0.001$; Table S1). The DM_{adp} of cvs. Sevenop, Kora and Jordane were significantly higher than cvs. Amba, Bamse and Chieftain ($P < 0.001$). Cultivar Jordane had the highest DM_{adp} of 5.89 t ha^{-1} and cv. Bamse the lowest of 3.99 t ha^{-1} in the control plots (Fig. 2). Hykor seemed to have DM_{adp} similar to the three highest yielding cultivars.

Total dry matter yield, that is sum of yields before, during and after the drought stress period

There was a difference in DM_{total} between the two soil types and for each of the grasses the DM_{total} was higher on loamy than on coarse sand (Fig. 4). Significant differences between the eight cultivars were seen ($P < 0.001$; Table S2). In addition, there was interaction between soil and cultivar ($P < 0.05$) primarily because of very low DM_{total} for *M. giganteus* on coarse sand. Across treatments, *M. lutarioriparius* had the highest DM_{total} on loamy sand (16.55 t ha^{-1}) and *M.* × *giganteus* the lowest on coarse sand (1.60 t ha^{-1}; Fig. 4). The yield of *M. lutarioriparius* was significantly higher than for all other grasses on both loamy and coarse sand. In addition, the cvs. Sevenop, Kora and Jordane had significantly higher DM_{total} than cvs. Bamse, Chieftain and *M.* × *giganteus* on both soil types. Furthermore, the yield of cv. Amba was lower than for cv. Kora on loamy sand, but higher than cv. Bamse and *M.* × *giganteus* on both soil types. The DM_{total} of cv. Hykor seemed to be at a similar level as cv. Kora on both soil types (Fig. 4). Interestingly, the average DM_{total} across cultivars and soil types was 10.39 and 10.28 t ha^{-1} for control and drought-treated plots, respectively, so the difference was very small and not significant (Table S2).

Water use efficiency

Cultivar Jordane had significantly higher WUE than all other cultivars except cv. Sevenop across soil types during the drought stress period in 2015 ($P < 0.05$; Fig. 5b). Across treatments and soil types cvs. Sevenop and Jordane had significantly higher WUE (3.12 g l^{-1} and 3.29 g l^{-1}, respectively) than the other cultivars, which had average values between 2.35 and 2.70 g l^{-1} ($P < 0.01$). Cultivar Hykor seemed to have an intermediate WUE (2.92 g l^{-1}; Fig. 5b). In 2014, cv. Sevenop also had WUE in the high end (3.60 g l^{-1}) but this was not significant ($P = 0.08$).

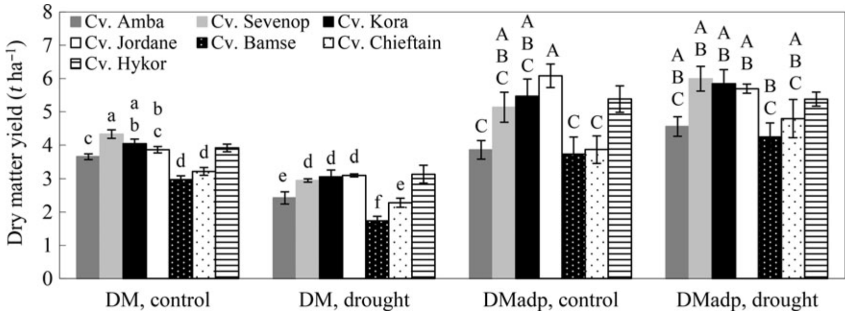

Fig. 2 Dry matter yields (DM) and DM after the drought period (DM_{adp}) of seven perennial grasses (cvs. Amba, Sevenop, Kora, Jordane, Bamse, Chieftain and Hykor) in control and drought-treated plots in 2015 as an average of two soil types. The DM was the production during the drought stress period from 21 May until 2 July. The DM_{adp} was the biomass produced after the drought stress period from 2 July until harvest on 7 October. No plots were drought stressed during this time. Error bars represent SE (*n* = 6). Cultivars with the same letter are not significantly different at the *P* = 0.05 level. Lower-case letters show the comparison for the drought stress period, and upper-case letters for the period after the drought stress treatment.

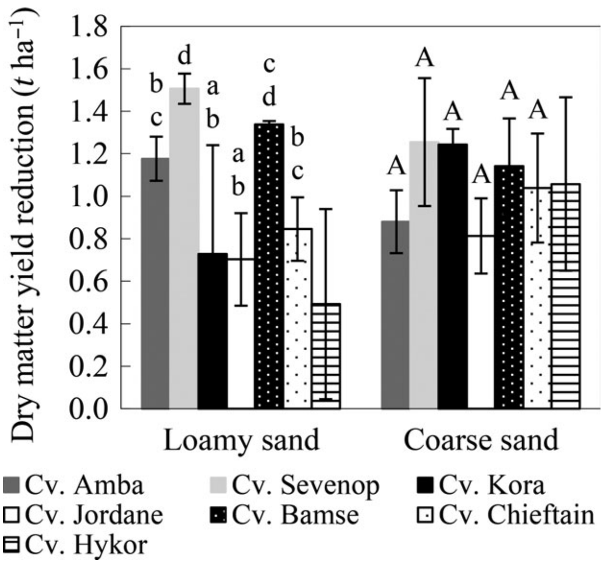

Fig. 3 Dry matter yield reduction in seven perennial grasses in drought-stressed plots compared to control plots on loamy and coarse sand during the drought treatment period in 2015. Error bars represent SE (*n* = 3). Cultivars with the same letter are not significantly different at the *P* = 0.05 level. Lower-case letters show the comparison for loamy sand, and upper-case letters for coarse sand.

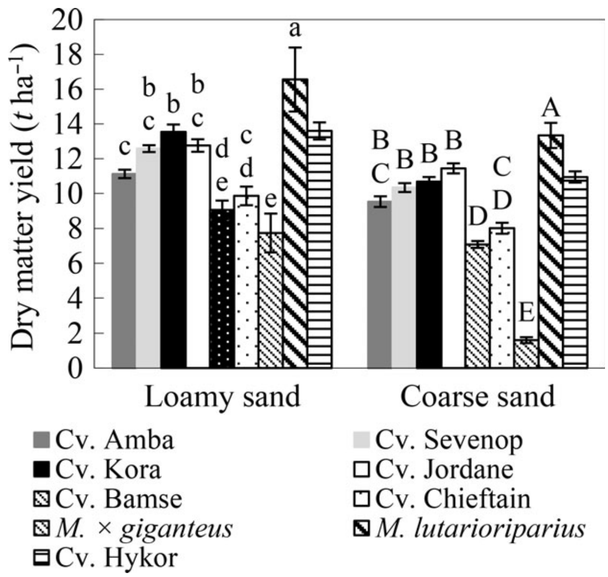

Fig. 4 Total dry matter yield (DM_{total}) of nine perennial grasses in the entire 2015 growing season. Average yields of control and drought-treated plots on loamy and coarse sand. Error bars represent SE (*n* = 6). Cultivars with the same letter are not significantly different at the *P* = 0.05 level. Lower-case letters show the comparison for loamy sand, and upper-case letters for coarse sand.

In 2015, significantly ($P < 0.01$; Table S1) higher average WUE was found in the drought-treated plots (2.84 g l^{-1}) compared to the controls (2.62 g l^{-1}). Such difference between treatments was also observed in 2014 (Fig. 5a) with WUE of 3.49 g l^{-1} and 3.15 g l^{-1} in drought-treated and control plots, respectively ($P < 0.05$; Fig. 5a).

The WUE_{total} also showed significant differences between cultivars ($P < 0.001$) and treatments ($P < 0.01$) as well as interaction between cultivar and soil ($P < 0.01$; Table S2). *Miscanthus lutarioriparius* had the highest WUE_{total} with an average of 3.61 g l^{-1} (Fig. 5b), which was significantly higher than all other grasses ($P < 0.001$). Cultivar Jordane had the second highest WUE_{total} of 2.69 g l^{-1}, and this was significantly higher than cvs. Amba, Bamse, Chieftain and *M.* × *giganteus* ($P < 0.01$). The highest values of WUE_{total} were found in the drought-treated plots and on loamy sand.

Carbon isotopic composition

Plants from drought-treated plots had a less negative $\delta^{13}C$ than those grown under irrigated conditions ($P < 0.001$; Fig. 6a). Within the drought-treated plots cv. Bamse had significantly higher $\delta^{13}C$ than cv. Chieftain ($P < 0.05$). Across soil types and treatments cv. Jordane showed a tendency to high $\delta^{13}C$ of −28.9‰ and cv. Chieftain to low with −29.6‰ ($P = 0.061$). Cultivar Hykor had similar $\delta^{13}C$ (−28.4‰) as found for cv. Jordane.

Leaf water potential

The Ψ_l measurements showed significant difference between treatments ($P < 0.001$; Table S1). As expected, the lowest Ψ_l was measured on the drought-treated plants (Fig. 7) with an average of −1.58 MPa compared to −0.84 MPa in control plots. The Ψ_l of the grasses across treatments were lower on coarse sand (−1.32 MPa) than loamy sand (−1.10 MPa). Similar observations were made in 2014 (data not shown).

The results also showed interaction between treatments and soil types ($P < 0.001$; Table S1), as the drought-treated grasses on coarse sand had lower average Ψ_l value than on loamy sand (−1.80 and −1.36 MPa, respectively), while similar for control plots (−0.83 and −0.84 MPa on loamy and coarse sand, respectively; data not shown).

Stomatal conductance

The average g_s for cv. Bamse across soil types and treatments was lower than for cvs. Sevenop and Jordane ($P < 0.05$; data not shown). In addition, cv. Chieftain had lower value than cv. Jordane. It seemed that the g_s of cv. Hykor was lower than of cv. Jordane, but higher than the other grasses. The g_s was lower in drought-treated (118 mmol m^{-2} s^{-1}) than irrigated (353 mmol m^{-2} s^{-1}) plants ($P < 0.001$), and there were indications that it was lower on coarse (203 mmol m^{-2} s^{-1}) compared to loamy sand (269 mmol m^{-2} s^{-1}; Fig. 7).

The analysis also showed a significant interaction between cultivars and treatments ($P < 0.05$; Table S1). Significant differences within the control plots were observed, such that cvs. Jordane and Sevenop had higher g_s (472 and 435 mmol m^{-2} s^{-1}, respectively) than cvs. Kora, Bamse and Chieftain (303, 232 and 305 mmol m^{-2} s^{-1}, respectively). Also cv. Amba had higher g_s (374 mmol m^{-2} s^{-1}) than cv. Bamse. The results suggested that the control plots of cv. Hykor also had a high g_s of 449 mmol m^{-2} s^{-1}.

Ratio vegetation index

When considering the development of the average RVI in 2015 of the seven C_3 grasses before and until one month after the drought treatment period had finished (Fig. 8a), it was observed that as the drought period progressed there was an increasing difference between the drought-treated and control plots with the highest RVI in control plots ($P < 0.001$). However, in the first month after termination of the drought treatment, where all plots were irrigated to full water capacity, the previously drought-stressed plots had the highest RVI. Such relations were supported by the preliminary

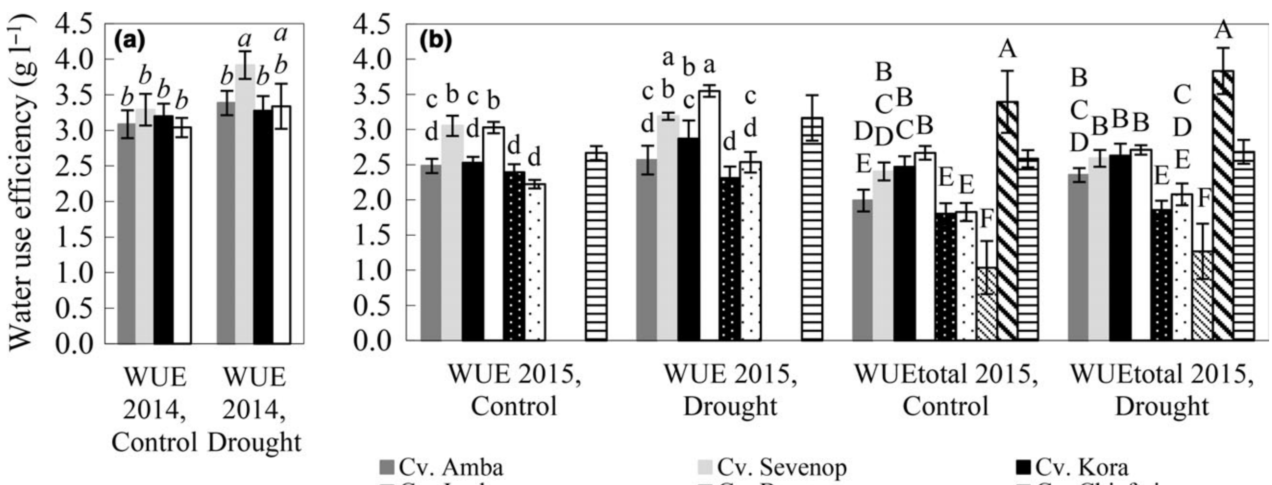

Fig. 5 Mean water use efficiency (WUE) across soil types in four perennial grasses in 2014 (a) and nine in 2015 (b). The WUE was measured based on the dry matter produced and the water used in the drought period (WUE) and the whole growing season (WUE_{total}). Error bars represent SE ($n = 6$). Cultivars with the same letter are not significantly different at the $P = 0.05$ level. Lower-case letters show the comparison for the drought stress period, and upper-case letters for the whole growing season.

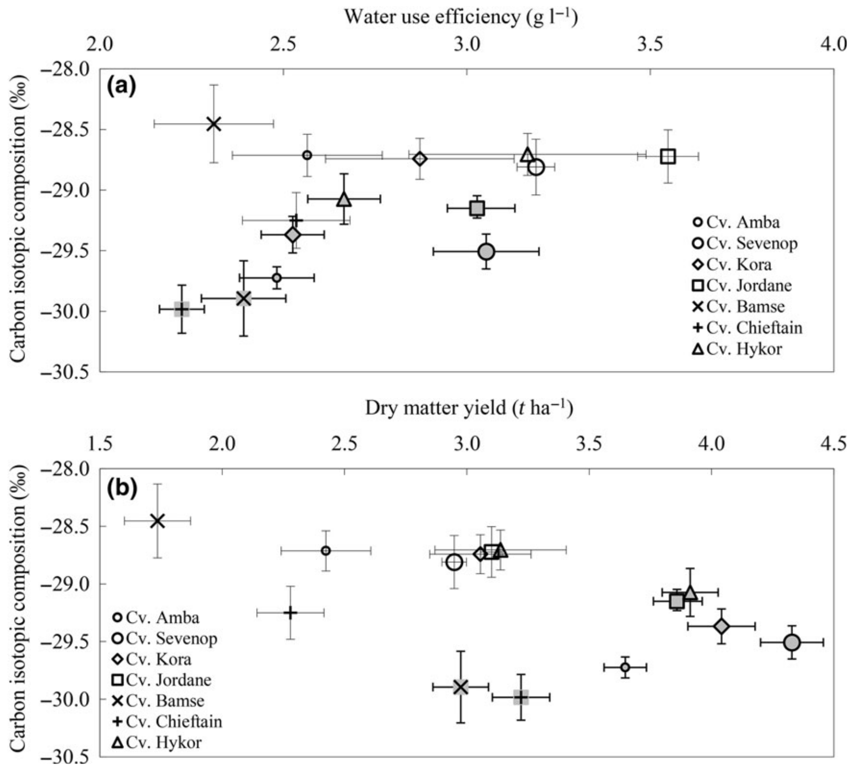

Fig. 6 The relationship between carbon isotopic composition and water use efficiency (a) and dry matter yield (b) of seven perennial grasses as an average of two soil types grown under control (irrigated, filled symbols) and drought (empty symbols) conditions in 2015. Error bars represent SE (n = 6).

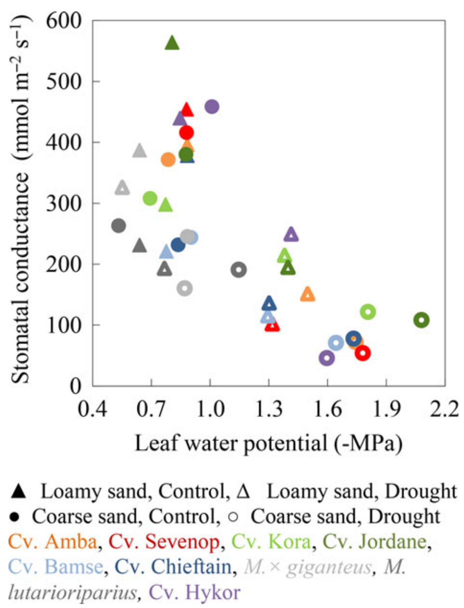

Fig. 7 The stomatal conductance (g_s) and leaf water potential (Ψ_l) for nine perennial grasses grown on loamy and coarse sand under drought-stressed and control (irrigated) conditions (n = 3).

experiment in 2014 (P < 0.001; Fig. 8b). Four weeks after termination of drought in 2015, control and drought-treated plots reached similar levels of RVI and then the trend was that the control plots had the highest RVI. In 2014, the previously drought-treated plots continued to be superior to control plots.

At harvest on 2 July in 2015 the RVI for cv. Amba was lower than cvs. Sevenop, Kora and Jordane across soil types and treatments (P < 0.01). The RVI of cv. Hykor seemed lower than the RVI of these three cultivars. In 2014, cv. Amba as well as cv. Sevenop had significantly lower RVI than cvs. Kora and Jordane (P < 0.001; data not shown). In both years, the RVI was lower in drought-treated compared to control plots (P < 0.001).

Intercepted photosynthetically active radiation

In 2015, higher IPAR during the drought treatment period were found in cvs. Sevenop, Kora and Jordane compared to cvs. Amba, Bamse and Chieftain (P < 0.001), as well as in cv. Amba compared to cvs. Bamse and Chieftain (P < 0.001), and in control compared to drought-stressed plots (P < 0.01; data not shown).

The $IPAR_{total}$ showed significant difference between cultivars and interaction between cultivars and soil types ($P < 0.001$; Table S2), but $IPAR_{total}$ was higher on loamy compared to coarse sand for all cultivars except for *M. lutarioriparius*. Across soil types and treatments, $IPAR_{total}$ was generally higher in the C_3 (from 809 to 983 MJ m^{-2}) compared to the C_4 plants (451 and 649 MJ m^{-2}). The highest value was observed in cv. Kora and the lowest in *M.* × *giganteus*. Hykor had a relatively high value (955 MJ m^{-2}).

Radiation use efficiency

The RUE was higher in control plots during the drought treatment period than in drought-treated plots ($P < 0.001$). Cultivar Sevenop had the highest RUE with 1.29 g MJ^{-1} and cv. Bamse the lowest with 1.01 g MJ^{-1}, implying that cv. Bamse was significantly lower than the other five C_3 grasses ($P < 0.05$) and cv. Amba was significantly lower than cv. Sevenop ($P < 0.01$).

Contrary to the RUE, there was no significant difference between treatments for the RUE_{total}, but for each of the grasses, the RUE_{total} was higher on loamy than on coarse sand (Fig. 9). There were differences between cultivars at each soil type ($P < 0.001$; Table S2), such that *M. lutarioriparius* had the highest RUE_{total} (2.57 g MJ^{-1}) of all grasses on loamy sand ($P < 0.001$) and *M.* × *giganteus* the lowest on coarse sand ($P < 0.001$; Fig. 9).

Correlation between variables

According to partial correlation coefficients, there was a positive correlation between g_s and $\delta^{13}C$ ($P < 0.01$; data not shown). It was found that Ψ_l was more negative and g_s was lower on coarse sand compared to loamy sand (Fig. 7) and that the cultivars with a high (less negative) average Ψ_l across soil types and treatments had a low g_s (data not shown). According to Pearson coefficient of correlation, this association was significant ($P < 0.01$). Furthermore, this analysis suggested positive, significant correlations between g_s and DM as well as WUE ($P < 0.05$) across soil types and treatments. In the control plots and across soil types and treatments, there were indications of positive associations between WUE and $\delta^{13}C$ ($P < 0.09$; Fig. 6a) and between DM and $\delta^{13}C$ ($P < 0.05$; Fig. 6b) when nonsignificant interactions were excluded from the analysis. Between DM and DM_{adp}, a positive correlation was observed ($P < 0.01$).

The partial correlation coefficients between DM and WUE as well as WUE and RUE were positive both

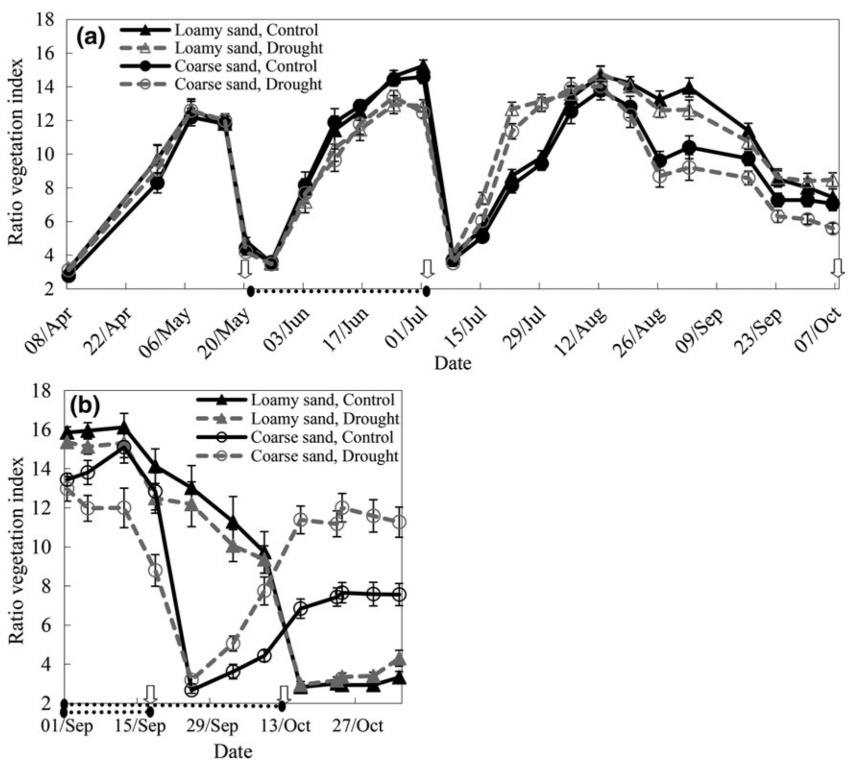

Fig. 8 The average ratio vegetation index (RVI) in control and drought-treated plots on loamy and coarse sand of seven cultivars (Amba, Sevenop, Kora, Jordane, Bamse, Chieftain and Hykor) in 2015 (a) and of four cultivars (Amba, Sevenop, Kora and Jordane) in 2014 (b). The grasses were cut on 19 May, 2 July and 7 October 2015 and on 19 September and 13 October 2014 on coarse and loamy sand, respectively (open arrows). The drought period was from 21 May until 2 July 2015, and from 8 August until 19 September and 13 October 2014 on coarse and loamy sand, respectively (black dotted line). Error bars represent SE (n = 21 in 2015 and n = 12 in 2014).

during the drought treatment period and for the whole growing season ($P < 0.001$). Furthermore, there was a positive, significant correlation between iPAR and RUE ($P < 0.001$), $iPAR_{total}$ and DM_{total} ($P < 0.01$) and between $iPAR_{total}$ and WUE_{total} ($P = 0.001$).

Discussion

Variation in yield, yield reduction and water use efficiency

Identification and further development of grasses with increased drought tolerance and WUE would be valuable for agricultural production under climate change. Here, the cvs. Kora and Jordane had the lowest decrease in DM on loamy sand during drought. Furthermore, the cvs. Sevenop, Kora, Jordane and Hykor produced higher DM than the other tested cultivars during drought treatment. This was not solely species dependent, which is equivalent to what has been reported previously (Reed *et al*., 2008). Moreover, we found that these four C_3 grasses also produced the highest yields when irrigated. Here, DM ranged between 12.5–13.6 and 9.5–11.5 t ha^{-1} on loamy and coarse sand, respectively. This is lower than harvested in field trials of cocksfoot, tall fescue and festulolium cultivars with yields between 15.2–20.4 and 15.3–17.7 t ha^{-1} on loamy and coarse sand, respectively (Manevski *et al*., 2017).

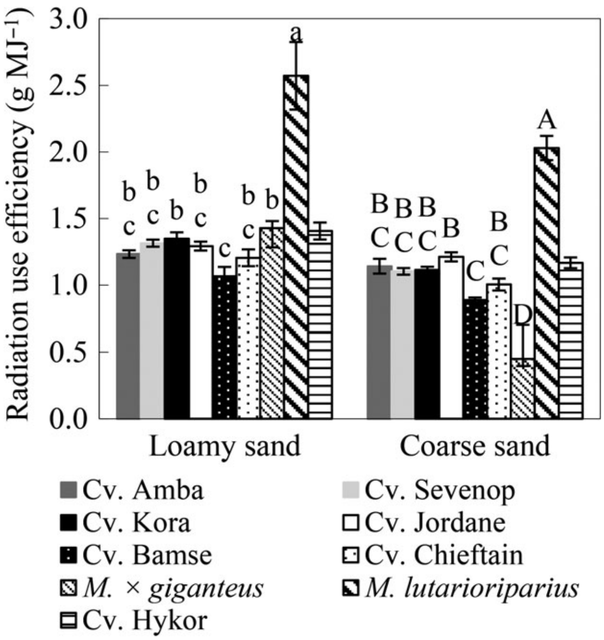

Fig. 9 Whole growing season radiation use efficiency (RUE_{total}) in nine perennial grasses in 2015. Error bars represent SE ($n = 6$). Cultivars with the same letter are not significantly different at the $P = 0.05$ level. Lower-case letters show the comparison for loamy sand and upper-case letters for coarse sand.

Still high shoot biomass production has been found in greenhouse experiments both after drought and irrigation, where cv. Sevenop was more productive than cvs. Kora and Jordane (Mårtensson *et al*., 2017). Similar observations of high productivity under different conditions have also been made by Rizza *et al*. (2004) in barley (*Hordeum vulgare* L.) who found that selection in favourable surroundings produced genotypes that also performed well in drought-stressed environments. Although this indicates that modern cultivars already contain some drought tolerance, targeted breeding for a high biomass production during drought stress might enhance this trait. It could be achieved by combining traditional breeding with improved methods developed within plant physiology, molecular genetics, molecular biology (Cattivelli *et al*., 2008) and modelling (Wollenweber *et al*., 2005). In maize, this has successfully been used to develop new hybrids with improved grain yield and yield stability in drought-prone environments (Cooper *et al*., 2014).

In addition to high DM, cvs. Sevenop and Jordane showed higher WUE than the other tested cultivars under both irrigated and drought-stressed conditions. Generally, DM was found to be positively correlated with WUE. Such a relation has previously been observed in other accessions of *D. glomerata* and *F. arundinacea* (Gulías *et al*., 2012). It has also been found that g_s decreased while WUE increased in drought-treated *D. glomerata*, which suggests that g_s might be used as an indicator of WUE under water-limited conditions (Gulías *et al*., 2012). However, we did not find such a negative association. On the contrary, we observed a positive correlation both within *D. glomerata* and across cultivars, indicating that these grasses benefit from keeping the stomata open and maintain a relatively high biomass production.

As opposed to the other grasses cv. Bamse did not increase WUE when water stressed, which indicated lack of drought adaptation ability. However, here the influence of only one-two drought events on DM and WUE was investigated, and the cultivars might react differently if grown under extreme or prolonged drought where ability to escape water scarcity may become more important. Clearly, we found differences between cultivars, but further experiments are needed to evaluate the impact of drought duration and severity on the performance of different grasses. Such experiments could include below-ground biomass production for interpretation of potential effects.

Carbon isotopic composition as a predictor of water use efficiency

Our results suggest that there may be a positive association between WUE and $\delta^{13}C$ in the C_3 grasses under well-watered conditions. Previous findings showed a similar relation when these grasses were grown both with and without lack of water (Zhu *et al.*, 2016) although the correlation between WUE and $\Delta^{13}C$ was negative because of different definitions of $\delta^{13}C$ and $\Delta^{13}C$. Such a relationship has been found in wheat (*Triticum aestivum* L.) experiments too (Rebetzke *et al.*, 2002). They also found $\Delta^{13}C$ useful for selecting wheat lines with higher aerial biomass and grain yield especially in dry environments. In our studies, there seemed to be a positive relation between DM and $\delta^{13}C$ in control plots. This trend was in accordance with former results in these grasses (Mårtensson *et al.*, 2017) where a negative correlation also was found between shoot biomass and $\Delta^{13}C$ in drought-treated plants. Previously, a positive correlation between grain yield and $\Delta^{13}C$ in spring wheat has been reported (Fischer *et al.*, 1998). This was also found by Xu *et al.* (2007) across environments and under water-stressed conditions. The relation between $\Delta^{13}C$ and yield can be positive, negative or neutral, depending on both genotype (species) and environment (season and location; Condon *et al.*, 2004; Chen *et al.*, 2011). Although this implies that it is complex to use $\Delta^{13}C$ and $\delta^{13}C$ as predictors of WUE and above-ground biomass production, our results suggest that $\delta^{13}C$ might be used as an indicator of WUE and DM under conditions where the grasses are not drought stressed.

Morphology, stomatal conductance and leaf water potential

It was observed that leaves on the drought-stressed plants of cv. Jordane were rolling (Fig. S2a,b). Such a strategy of minimizing the effective leaf area to reduce water loss as described by Ludlow (1989) seems advantageous compared to the response observed in the cv. Amba where the leaves were hanging limply down (Fig. S3a,b). However, this morphological response is not the only reason for better performance of cv. Jordane concerning WUE and DM during drought as cv. Sevenop, which showed similar response as cv. Amba, exhibited higher WUE and DM than cv. Amba.

Loss of tissue turgidity as seen in cvs. Amba and Sevenop during drought treatment was in line with their more closed stomata observed through the lower g_s that was measured in these cultivars compared to cvs. Kora and Jordane. All the grasses – though at different levels – responded to drought stress and saved water by closing the stomata as the g_s was lower in the drought-treated compared to the control plots. Overall, we found a negative correlation between g_s and Ψ_l. This indicates that evaluation of drought stress in grasses might be performed by assessment of g_s, which is easier to measure than Ψ_l. Previously, g_s has been found to be a good reference parameter to reflect drought intensity in C_3 plants (Medrano *et al.*, 2002). Application of g_s as a predictor of drought stress might be utilized even further as it might be estimated indirectly through modern thermal infrared imaging (Prashar & Jones, 2016). This could be a high-throughput method for assessment of g_s and phenotyping of drought stress, and thereby a tool for breeding of future cultivars with improved drought tolerance (Lootens *et al.*, 2016; Prashar & Jones, 2016).

The g_s might also be used as an indicator of biomass production potential in these perennial grasses due to the significant correlation between g_s and DM. However, Ψ_l is apparently not useful for this purpose, as no correlation between Ψ_l and DM was found. A similar finding was made in Napier grass (*Pennisetum purpureum*) where measurements of Ψ_l were not adequate for selecting cultivars for dry environments (Mwendia *et al.*, 2013).

Compensatory growth after drought

In both years, the drought treatment resulted in a significantly reduced DM in the drought-stressed grasses compared to control. Contrary to this, in the period after drought the highest yields were produced in the previously drought-treated plots. Similar observations have been made in temperate forage grasses (Hofer *et al.*, 2016). In accordance with the increased yields in our study, a higher RVI was measured in the first weeks after irrigation of previously drought-stressed plots. This indicates that when grasses, that had been drought-stressed, were re-irrigated they managed to utilize the soil N resources (Hofer *et al.*, 2016) or they had stored reserves ready for use, and were able to compensate for the lower above-ground yield produced during drought. The reserves might be stored in roots and stubble, as it has previously been observed that recovery after drought was associated with a large pool size of fructans with a high degree of polymerization in entire tiller bases (Volaire *et al.*, 1998a). Furthermore, it has been found that dry matter can be allocated to roots during stress as a possible short-term cost for shoot growth (Ludlow, 1989). We observed no significant effect of drought treatments on the DM_{total} and RUE_{total} for the whole growing season. Additionally, the WUE was higher in drought-treated compared to control plots, not only during the drought stress period but also for the whole growing season, so drought at the investigated level seems to be useful for saving water. This may be different in more severe drought conditions with prolonged drought or higher evaporation demand than in the rather cool Danish climate.

Comparison of C_3 and C_4 grasses

During the whole growing season, the cultivar that produced the lowest DM was the C_4 grass

M. × *giganteus*, although the yield of 7.7 t ha^{-1} on loamy sand was higher than previously observed (3.4 Mg ha^{-1}) in the second year after planting on this soil type and location (Manevski *et al*., 2017). This is most likely due to a bad establishment as a slow establishment rate is a major problem in this species especially in cool areas (Clifton-Brown *et al*., 2001), and it is suggested to be partly because of severe winter death (Larsen *et al*., 2014). In warmer climatic conditions, yields of 25.5 t ha^{-1} is found in the second growth year (Clifton-Brown *et al*., 2001). Low overwintering survival has also been observed in the other C_4 grass, *M. lutarioriparius*, at very low temperatures (down to −22.9 °C). Otherwise, this species has been reported to have superior characteristics for good crop establishment (Yan *et al*., 2012). In the present study, *M. lutarioriparius* produced the highest DM_{total} of all grasses, which aligns with previous projections of a high biomass production in this perennial grass (Yan *et al*., 2012, 2015).

We found highly significant correlations between DM_{total} and $IPAR_{total}$. Even though the $IPAR_{total}$ was lower in the C_4 than the C_3 grasses, which is consistent with reported results (Manevski *et al*., 2017), the DM_{total} was larger leading to an RUE_{total} almost twice as high in *M. lutarioriparius* compared to the other tested grasses. The RUE in C_4 grass species has been observed to be less influenced by resource limitations, like water availability, than in C_3 grasses (Cristiano *et al*., 2015). The restrictive effect on C_3 grasses was also found in our studies as the measurements during the drought treatment period showed that RUE was affected negatively by water limitation, even though we did not evaluate root biomass, which Cristiano *et al*. (2015) found important.

The Ψ_l was lower in drought-stressed *M. lutarioriparius* than in control plants during the drought treatment, but not as low as in C_3 plants. Furthermore, we found a higher g_s in drought-treated miscanthus (except *M. lutarioriparius* on loamy sand) than in C_3 grasses, even though the C_4 species were irrigated with less water (Table 3) as they used less than any of the C_3 species (Fig. 1a,b). This could have been due to water uptake from deeper soil layers if the miscanthus plants had deep rooting. It has been found that *M.* × *giganteus* can have a root system down to 300 cm (Ferchaud *et al*., 2015). However, this seems not to be the reason here as none of the grasses used water at 100 cm depth according to our measurements of soil water use (Fig. 1). Therefore, it appears that C_4 grasses, in particular *M. lutarioriparius*, were less affected by drought than C_3 grasses. This is in accordance with previous studies, which found that C_4 grasses have an advantage compared to C_3 grasses under field conditions during drought (Taylor *et al*., 2014). This was partly caused by higher g_s in C_4 species, which is in agreement with our finding. The increased g_s together with higher hydrodynamic gradients when grown under water-limited conditions seemed to enhance the photosynthetic differences in C_4 compared to C_3 species (Taylor *et al*., 2014).

Yan *et al*. (2015) also observed a high photosynthetic rate – and a high WUE – of *M. lutarioriparius* in areas with less precipitation and low temperature compared to warmer and wetter environments, and therefore, they found this species useful as an energy crop in semiarid and cool regions. Generally, C_4 plants are considered to have enhanced WUE compared to C_3 plants (Way *et al*., 2014). High WUE is in accordance with our results as *M. lutarioriparius* had the highest WUE_{total} among all the tested grasses. This higher WUE compared to that of C_3 grasses might partly be explained by the enhanced efficiency of radiation use in this species as the correlation between WUE_{total} and RUE_{total} was strongly significant.

If a high biomass production is the trait of highest value, the C_4 perennial grass *M. lutarioriparius* seems very interesting, as it produced the highest yields on both soil types. It might also have potential for cultivation in water deficient areas as it used the least amount of water and at the same time had the highest WUE_{total} both in drought-stressed and control plots.

The C_3 cvs. Sevenop, Kora and Jordane grew well on both soil types and produced DM at equal levels, which were higher than the other three tested C_3 grasses during drought. This might be partly due to high WUE and IPAR during drought, indicating that they are less affected by drought and transform the incoming radiation into biomass more effectively than the other tested cultivars. These findings are useful for selection of cultivars with superior drought tolerance that simultaneously maintain a high yield potential. Further investigations are needed for development of cultivars which do not only have a high drought tolerance but also produce biomass with sufficient quality for biorefining and bioenergy during drought stress, for example on water constrained marginal land.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Lysimeter layout and randomisation of grass cultivars.
Figure S2. Drought stressed and control (irrigated) plots of *Festuca arundinacea* cv. Jordane.
Figure S3. Drought stressed and control (irrigated) plots of *Dactylis glomerata* cv. Amba.
Table S1. Results from analysis of variance of measured variables in six perennial grasses.
Table S2. Results from analysis of variance of variables measured for the whole growing season in eight perennial grasses.