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Yield performance of 14 novel inter- and intra-species *Miscanthus* hybrids across Europe

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Abstract

Miscanthus, a C4 perennial rhizomatous grass from Asia is a leading candidate for the supply of sustainable biomass needed to grow the bioeconomy. European *Miscanthus* breeding programmes have recently produced a new range of seeded hybrids with the objective of increasing scalability to large acreages limited by current clonal propagation. For the EU-GRACE project, new replicated field trials were established in seven locations across Europe in 2018 with eight intraspecific *M. sinensis* hybrids (*sin*×*sin*) and six *M. sacchariflorus*×*M. sinensis* (*sac*×*sin*) from Dutch and UK breeding programmes, respectively, with clonal *Miscanthus*×*giganteus*. The planting density of the *sin*×*sin* was double that of *sac*×*sin* (30,000 & 15,000 plants ha^{-1}), creating commercially relevant upscaling comparisons between systems. Over the first 3 years, the establishment depended

on location and hybrid. The mature *sin*×*sin* hybrids formed tight tufts of shoots up to 2.5 m tall which flower and senesce earlier than the taller *sac*×*sin* hybrids. Following the third growing season, the highest yields were recorded in Northern Italy at a low altitude (average 13.7 (max 21) Mg DM ha^{-1}) and the lowest yielding was on the industrially damaged marginal land site in Northern France (average 7.0 (max 10) Mg DM ha^{-1}). Moisture contents at spring harvest were lowest in Croatia (21.7%) and highest in Wales, UK (41.6%). Overall, lower moisture contents at harvest, which are highly desirable for transport, storage and for most end-use applications, were found in *sin*×*sin* hybrids than *sac*×*sin* (30% and 40%, respectively). Yield depended on climate interactions with the hybrid and their associated planting systems. The *sin*×*sin* hybrids appeared better adapted to northern Europe and *sac*×*sin* hybrids to southern Europe. Longer-term yield observations over crop lifespans will be needed to explore the biological (yield persistence) and economic costs and benefits of the different hybrid systems.

KEYWORDS

biomass, *M. sinensis*, *Miscanthus*, *Miscanthus*×*giganteus*, *Miscanthus* seeded hybrids, multi-location field trials, perennial biomass crop

1 | INTRODUCTION

New clonally propagated and seeded hybrids of *Miscanthus* are being developed in Europe as a future source of sustainable biomass for the bioeconomy, rural diversification and resilience to address climate changes and global energy security (Clifton-Brown et al., 2017). *Miscanthus*, a grass with C_4 photosynthesis from Asia, is an ideal perennial biomass crop because it combines high yield with high nutrient and water-use efficiency (Jones, 2019). *Miscanthus* for biomass in Europe is grown commercially from clonal rhizomes but low multiplication rates mean this is slow and costly to upscale (Xue et al., 2015). Rapid upscaling is needed in the face of recent requirements for huge supplies of biomass for the bioeconomy. Also demand from bioenergy and BECCS (Bioenergy with carbon capture and storage) which could provide negative greenhouse gas emission technologies to meet climate change objectives (CCC, 2018). To avoid conflicts with food production, perennial biomass crops such as *Miscanthus* need to grow in a wide range of climates on a wide range of land types including those classed as marginal land (Lewandowski et al., 2016; McCalmont et al., 2017; Valentine et al., 2012; von Cossel et al., 2019). Land availability estimates for perennial biomass crops in Europe are hard to predict because of the complex global interactions with food, energy and water. Estimates range from 13 (Don et al., 2012) to 58 Mha (Gerwin et al., 2018), drawing on experience from the set-aside policies in the early 1990s (Fischer et al., 2010). Ultimately, the land area depends on environmental and economic co-benefits compatible with many of the objectives of Common Agricultural Policy and the Sustainable Development Goals. The seed-based hybrid strategy has been pursued to develop the technology need to ramp up planting areas beyond current levels of several hundred to several thousand hectares per year. To this end, eight seed-based intraspecies *M. sinensis*×*M. sinensis* (*sin*×*sin*) hybrids bred at Wageningen University, four novel intraspecies *M. sacchariflorus*×*M. sinensis* (*sac*×*sin*) hybrids bred in Aberystwyth University, and two commercial hybrids *M*×*g* (Greef & Deuter, 1993; Hodkinson & Renvoize, 2001) and TV1 were planted in 2018 at seven sites in six countries on a wide range of latitudes following an approximate North-East to South-West transect across Europe (Figure 1). At each site, there are differences in temperature, photoperiod and water availability, all of which affect growth potential.

Early flowering time in lower latitude sites can negatively affect the yield (Clifton-Brown & Lewandowski, 2002; Gauder et al., 2012; Jensen et al., 2013). On the other hand, earlier flowering times can improve biomass quality by triggering active senescence before temperatures fall in autumn with better nutrient remobilisation from the shoot to the rhizome and convey cold resistance (Jensen et al., 2017; Lewandowski et al., 2003). Therefore, flowering time is a highly selected trait in *Miscanthus* breeding (Clifton-Brown, Harfouche, et al., 2019) and differentiates hybrids within environment. *M. sinensis* flowering is

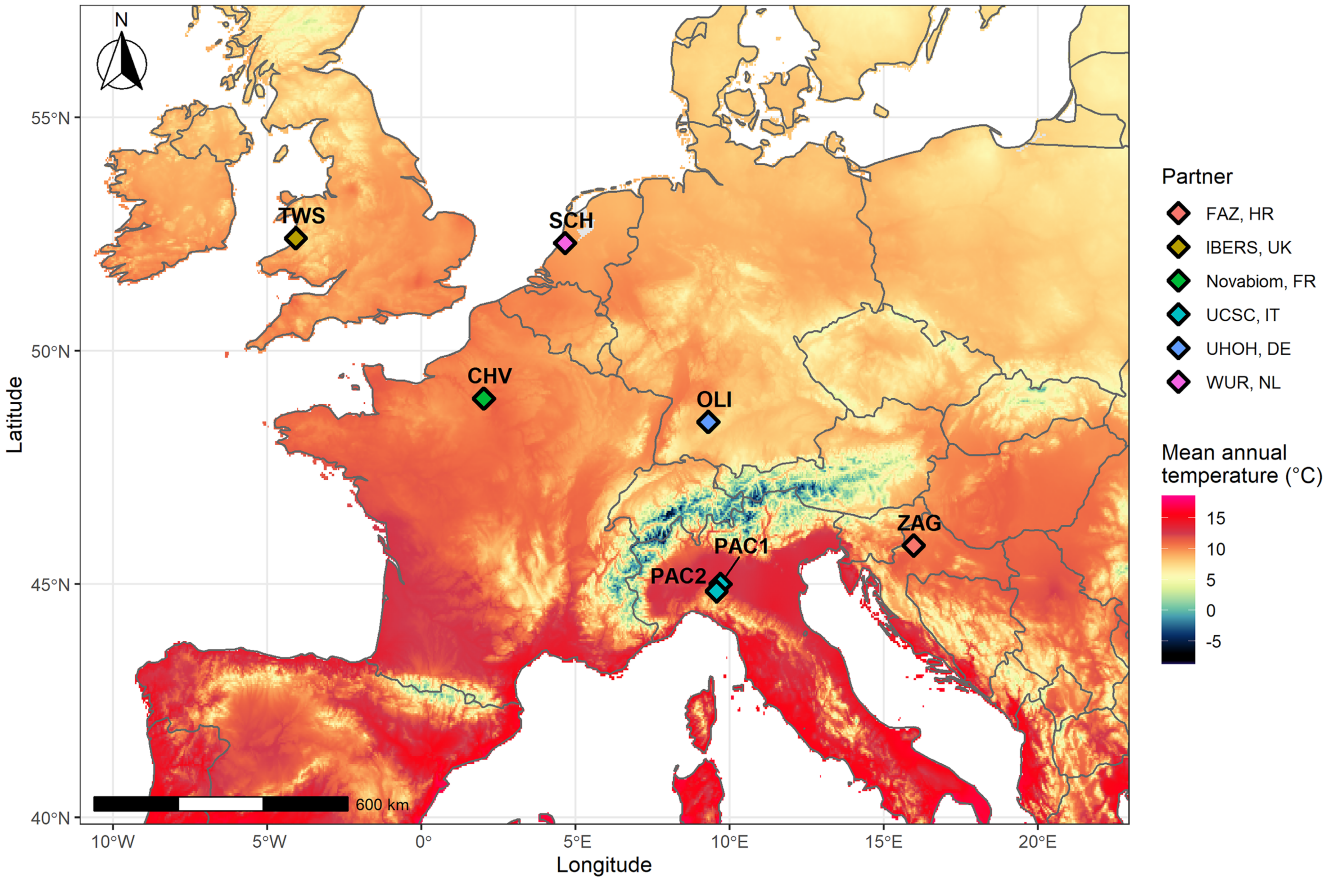

FIGURE 1 Map of the sites used, over a range of European environments partner organisations by colour. Map coloured by mean annual temperature 1970–2000 (Fick & Hijmans, 2017). Sites in order of decreasing latitude: TWS (Aberystwyth, Wales, UK) [Aberystwyth University, IBERS], SCH (near Amsterdam, Netherlands) [Wageningen University], CHV (near Paris, France), OLI (near Stuttgart, Germany) [University of Hohenheim], ZAG (Zagreb, Croatia) [University of Zagreb], PAC 1 & 2 (Po Valley, Italy) [Università Cattolica del Sacro Cuore].

normally day-length insensitive, while *M. sacchariflorus* generally requires short days below 12 h to trigger flowering (Jensen et al., 2013). A short-day requirement in *sac*×*sin* hybrids may result in later or absent flowering compared to *sin*×*sin* hybrids. Late or nonflowering hybrids reduces concern of invasiveness because plants unable to produce seed before winter frosts (Hastings et al., 2017).

The overarching aim of this multi-location trial was to determine which *Miscanthus* hybrid types (with their associated density of planting) produced the highest yield in the first 3 years after planting in seven different locations on a wide environmental gradient spanning from North-western to Mediterranean Europe. We expected as general trends that (i) the *sin*×*sin* hybrids planted at double the density of the *sac*×*sin* hybrids would yield highest in the first and second years, but this difference would shrink in the third year as the plants mature; (ii) *sin*×*sin* which flower early would be better adapted to cooler climates; (iii) these general trends would be modulated by the specific local conditions and (iv) the same few hybrids including *M*×*g* would excel across climates.

2 | MATERIALS AND METHODS

2.1 | Trial sites

At seven locations across Europe, field trials were established within the EU-BBI funded 'GRowing Advanced industrial Crops on marginal lands for biorEfineries' (GRACE) project. These are arranged in an approximate North-East to South-West transect across Europe (Figure 1). The climates across these sites are diverse, as TWS (Trawsgoed) and SCH (Schiphol) are maritime, CHV (Chanteloup) and OLI (Oberer Lindenhof) are continental, and PAC (Piacenza) and ZAG (Zagreb) are Mediterranean. All the sites had 675 ± 200 mm of average yearly precipitation over the trial years 2018–2021,

apart from TWS with 1311 mm. The coldest sites are OLI in Germany and TWS in the UK. TWS and CHV had the least plant available water, although TWS has more than twice the annual precipitation of CHV. While OLI, ZAG and PAC1 combine high plant available water with over 800 mm of rain. Trial site soil type, location and marginality factors are presented in Table S1.

TWS (Trawsgoed near Aberystwyth in Wales, UK, 52°24′59.8″N, 4°04′02.6″W altitude 72 m) is located 12 km from the Welsh coast. The site has a stony sandy loam with lead contamination. The position of the site at the bottom of a steep slope at the junction of two valleys allows it to be sheltered from cold winds but can also subject the site to late frosts (e.g. mid-May). This site has high average precipitation over course of the trial, 1311 mm annually, and the lowest plant available water (50 mm). Previous use of the trial area was long-term improved grassland either for grazing or silage production, this was prepared in spring with ploughing and rotovating.

SCH (Schiphol near Amsterdam, Netherlands 52°18′45.2″N, 4°39′55.3″E altitude −4.5 m) is bordered by a highway and close to Schiphol international airport runway, with moderate average annual precipitation (832 mm). This site has calcareous loamy soil varying from silty to clayey, providing reasonable plant available water (147 mm). The soil has low available phosphorus (1 mg kg^{-1}) and an extremely low C:N ratio, also with some metal contamination, particularly magnesium. The land was originally a lake before becoming a polder. It was then extensively farmed for 150 years most recently winter wheat (*Triticum aestivum*).

CHV (Chanteloup near Paris, France, 48°58′34.9″N, 2°01′57.9″E altitude 42 m) has a lot of contamination from past activity on the site. Paris sewage waters have been used for over 100 years by fruit and vegetable growers for irrigation on sandy soils. A variety of heavy metals has added up over the years primarily Pb (lead), Zn (Zink) and Ca (Calcium) but also Cu (Copper), a problem that was enhanced by the wide usage of copper sulphate as a fungicide. In the 1940s, a sewage plant was added and by 2000 vegetable production was forbidden by law. Following this, there has been a period of neglect creating a vast area of fallow land colonised by itinerant communities, due to the vicinity of Paris. Also, after the second world war, contamination came from the heavy industry established in the close municipalities, for example, car manufacturing, power plants and aeronautic industry. The loamy sandy soil means water retention is low (second-lowest plant available water 67 mm), combined with the low annual precipitation of 537 mm which could make it hard to grow *Miscanthus* without irrigation. The general neglect of this area induced the proliferation of rabbits, rendering the cultivation impossible without electric netting around the trial plots. The high criminality in the area led to repeated vandalism of the rabbit fences, planting equipment, etc., which could also be considered an extra marginality factor at the Chanteloup site.

OLI (Oberer Lindenhof near Stuttgart, Germany 48°28′42.1″N, 9°18′41.0″E altitude 706 m) is located on the Swabian Alb, which is characterised by limestone providing good drainage. Due to its high altitude, the site has the shortest vegetation period of the sites, with late frosts in spring (until the end of May) and early frost in autumn (October). Over winter, it snows regularly, and temperatures as low as −20°C are reached. Due to this, the site is used as a cold tolerance testing station. Otherwise, the site has good precipitation (863 mm) and reasonable plant available water (148 mm) in its clay loam soil. Before the planting of *Miscanthus*, annual arable crops within a crop rotation were planted on the field and it was regularly fertilised with horse manure.

ZAG (Zagreb, Croatia 45°49′47.9″N, 15°58′33.5″E altitude 117 m) has a silt loam with good plant available water (158 mm) and the second-highest annual precipitation (891 mm), despite this the site can suffer from periods of drought in summer. The soil was marginal in terms of its productivity because of excessive water retention (pseudogley) with poor enzymatic activity, as well as acidity. The site was arable land before the planting of *Miscanthus* recently oil seed rape, cereals and lenses were cultivated.

PAC1 and PAC2 (Piacenza, Italy): The two sites represent a difference in altitude and former land use; the lowland site PAC1 (near Cerzoo [45°00′11.70″N, 9°42′35.4″E altitude 73 m]) being more conducive to *Miscanthus* growth while the upland site (PAC2, near Chiulano [44°50′40.32″N, 9°35′04.9″E altitude 578 m]) had stronger winds and lower temperatures but similar mean annual precipitation (820 and 804 mm, respectively). Both sites were on a clay loam soil with alkaline reaction with PAC1 having a high plant available water (166 mm) and PAC2 a lower (140 mm). The sites represent two different types of marginalities: PAC1 soil is characterised by a low organic carbon content (0.62%) and a high degree of compaction (soil bulk density 1.37 g cm^{-3}), while PAC2 is located on the top of a hill and considered marginal for its position within the agricultural landscape. PAC1 located in the Po river valley hosted a decadal crop rotation with cereals for animal feed while PAC2 has been established on a 15-year-old meadow. PAC1 was the warmest of all sites, while PAC2 had much lower thermal time. PAC2 was less often measured when compared to all other sites but was included as this could be an important type of site for retaining soil.

2.2 | Environmental conditions

At each site, an automatic station (Delta-T GP1, Cambridge, UK) was installed during the establishment phase to measure crop modelling relevant climatic parameters hourly: air temperature, humidity, rainfall, solar radiation and wind run at 2 m. Each station was connected through mobile data networks to the DeltaLINK-Cloud. Near real-time data were centrally visually checked at least weekly. Where measurements diverged from expected trends site operators were alerted to make sensor checks. Faulty sensors were repaired or replaced as quickly as possible to minimise data gaps. Where erroneous data was deleted, gaps were filled with a set of procedures similar to those established in the CarboEurope community (Moffat et al., 2007). Briefly, for short gaps up to a few days, simple running means from the hours before and after the gap were used to fill gaps. Large gaps were filled using data from nearby weather stations. Nearby stations were also used to compute the 10 year (long term) historical air temperature, rainfall and other parameters if measured (Table S2).

Potential evaporation was calculated using an excel version of the Penman-Monteith equation (Hess & Stephens, 1993) using daily radiation, temperature, humidity and windspeed. Soil moisture deficits were computed from the balance of actual evapotranspiration and rainfall in the MISCANFOR model (Hastings et al., 2009). This was also calculated with soil water support using the method by Shepherd et al. (2020). In the autumn 2017, before land conversion began at the seven sites, four 83 mm diameter cores to maximum of 1 m depth were taken from across the field site using an Eijelkampf tube corer (Eijkelkamp Soil & Water). The extracted cores were segmented into the following depth sections (0–10, 10–20, 20–30, 30–60 and 60–100 cm) in the field and placed in zip-loc bags. In the laboratory, 28 cores × 5 depth segments were weighed in the tared bags to determine the fresh weight. The samples were air-dried between 15 and 30°C in a laboratory or glasshouse in the bags which were rolled back like a sock. As the soil samples dried, the soil was crumbled by hand to speed the drying to near constant weight over several weeks. All samples were brought to IBERS UK. Roots were removed and the section samples was sieved to 2 mm. The resultant stone and fine fractions were weighed. The fine fractions were subsampled and used in IBERS (Aberystwyth University) to determine soil texture using hand analysis. Textures and bulk densities and depths were used to determine plant available water by a pedo-transfer functions (Campbell). Drought periods were identified when daily estimates of soil moisture deficit fell below the plant available water. Water support was added to some sites with evidence of ground water (Shepherd et al., 2022).

2.3 | Hybrid details

Eight *M. sinensis* × *M. sinensis* (*sin* × *sin*, GRC 1–8 (seeded)) intraspecific hybrids and six *M. sacchariflorus* × *M. sinensis* (*sac* × *sin*, GRC 9 (standard clonal *M.* × *giganteus* (*M* × *g*)), GRC 10, 11, 13 and 14 (seeded) and GRC 15 (TV1 clonal)) interspecific hybrids were selected from the *Miscanthus* breeding programme at Wageningen and Aberystwyth, respectively (Table 1). The justifications for the inclusion of these 14 hybrids in these GRACE trials were (1) to identify which hybrids combine the right traits and are ready for further development for commercial upscaling and deployment and (2) to inform breeders on trait combinations required for new seed-based hybrids.

The *sin* × *sin* hybrids have high stem numbers per plant which are thinner and shorter than that of the *sac* × *sin* hybrids. This morphological difference arises from reduced compact rhizomes in *sin* × *sin* hybrids and larger and more creeping rhizomes in the *sac* × *sin* ones. Therefore, to maximise yield potential, the *sin* × *sin*

TABLE 1 Details of the type of hybrids used and origin.

GRC number	Parental species (female × male)	Planting method	Supplier	Planting density
1–8	Intraspecies *M. sinensis* × *M. sinensis* (*sin* × *sin*)	Seed-based plug	Wageningen breeding program	3 plants m^{-2}
9	Wild Interspecies *M. sacchariflorus* × *M. sinensis* (*M* × *g*)	Rhizome cloning	Terravesta (Lincoln, UK) and Novabiom (Champhol, France)	1.5 plants m^{-2}
10–11 & 13–14	Interspecies *M. sacchariflorus* × *M. sinensis* (*sac* × *sin*)	Seed-based plug	Aberystwyth breeding program	1.5 plants m^{-2}
15	Interspecies *M. sacchariflorus* × *M. sinensis* (TV1)	Rhizome cloning	Terravesta (Lincoln, UK)	1.5 plants m^{-2}

hybrids were planted at 30,000 plants ha^{-1} (3 plants m^{-2}) double the density of the *sac*×*sin* hybrid planting 15,000 plants ha^{-1} (1.5 plants m^{-2}). *M. sinensis* bred for a northern climate can produce capable yields to *M*×*g* at northern latitudes (Clark et al., 2019; Fonteyne et al., 2016; Lewandowski et al., 2000). The *sac*×*sin* hybrids tend not flower at colder northerly locations in Europe, which may increase yield (Jensen et al., 2013; Zhao et al., 2013) but also could increase the moisture content and decrease senescence (Clifton-Brown & Lewandowski, 2002). Less compact *sac*×*sin* hybrids can be subdivided into clonal hybrids and seed-based hybrids. First, the clonally propagated *sac*×*sin* hybrids consist of the naturally occurring and widely used genotype, *M*×*g* which was propagated by Novabiom (Champhol, France) for the sites in the Netherlands, France, and Italy and by Terravesta Ltd (Lincoln, UK) for the fields in UK, Germany and Croatia. The other clonal interspecies hybrid used was GRC 15 produced by Terravesta Ltd. Second, the seed-based hybrids (GRC 10–14) are bred from the crossing of *M. sacchariflorus* and *M. sinensis* (Table 1), seed taken from the *M. sacchariflorus* side of the crosses and grown by Bells Horticultural Ltd (Boston, UK). All *sin*×*sin* hybrids (GRC 1–8) were provided as plug plants produced by Wageningen University. These seeded hybrids were sown into compost filled plugs in late January and grown under glasshouse conditions until late April 2018.

2.4 | Field planting

Each site's 0.6 ha trial was split into four replicate blocks, aligned with any known environmental gradients, with 14 randomised plots. These large (96 m^2) plots contained either 132 (*sac*×*sin*, 1.5 plants m^{-2}) or 264 (*sin*×*sin*, 3 plants m^{-2}) hand-planted plug plants (module grown seedlings) or rhizomes (Figure 2). GRC 9 (*M*×*g*) was not planted in SCH due to poor-quality rhizomes and the field design at PAC1 was arranged to make a longer field rather than a square field as in the other sites. Both PAC sites reduced the plot size due to a shortage of plugs, a border remind around the harvest area. Before planting in early 2018, the soils at each site were herbicide sprayed and inversion ploughed. Immediately before planting, the soil was 'worked' into a fine tilth to maximise the plug plant-to-soil hydraulic contact (Table S3). This was particularly important as the plugs were small. The *sin*×*sin* plugs plants were thinner and shorter (13 cm^3) compared to the *sac*×*sin* (35 cm^3).

The *sin*×*sin* hybrids were transported to the field sites as 'plugs-out-of-trays' in cardboard boxes, while the seeded *sac*×*sin* hybrids used a 'plugs-in-trays' system, using forklift compatible wooden crates. Even though the crates were film wrapped, the 'plugs-in-trays' method suffered more with drying during transportation. The crate 'plug-in-tray' transportation system, while in principle scalable to thousands of hectares, needs further development to prevent dehydration weakening plugs before

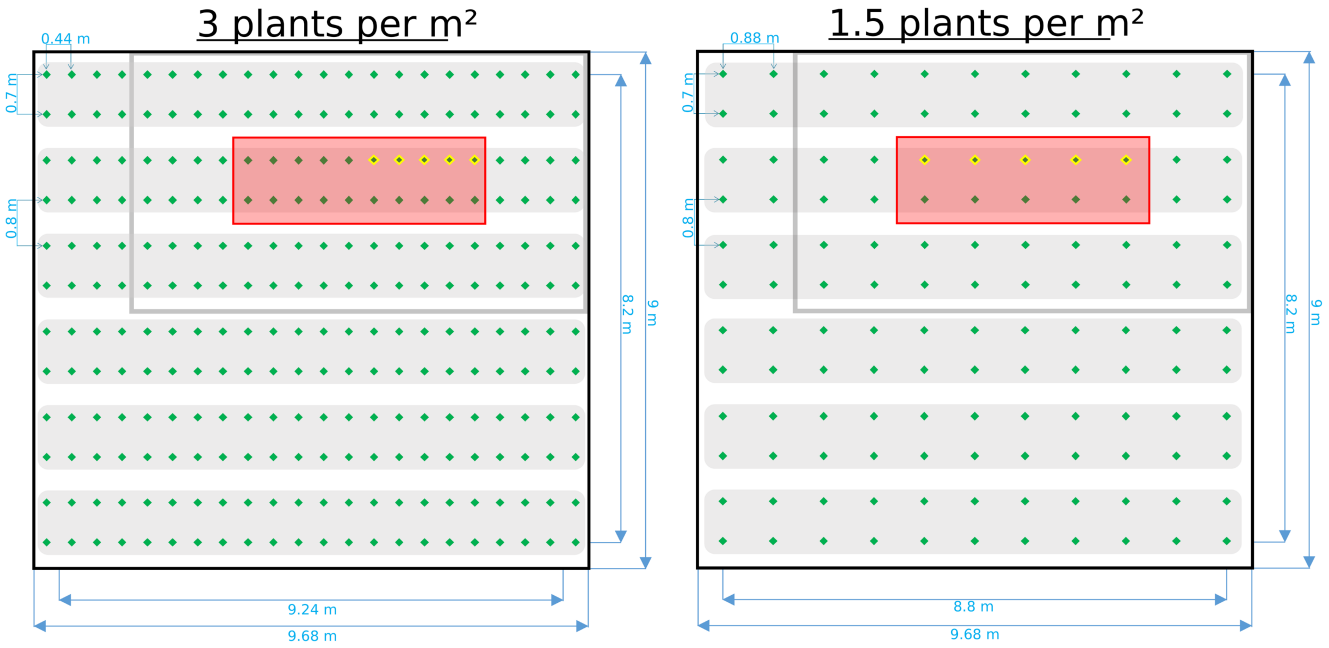

FIGURE 2 Plot dimensions and measurement positions for plots planted at 3 (GRC 1–8) and 1.5 (GRC 9–11, 13–15) plants m^{-2}. The harvest areas are highlighted in red, with individual marked plants in yellow, grey box indicates border area. Both PAC sites limited planting to eight rows, also the light grey area showing shows mulch film application at the TWS and OLI sites.

transplanting. Plugs were planted by hand on machine operable spacings and watered with about 2 L to help facilitate hydraulic contact between the plug and soil to prevent plug desiccation and death.

In the same week as the trials were planted at TWS and OLI, a mixture of soil acting herbicides were applied to inhibit the growth of weed seedlings, after which the plug plants of the seeded hybrids were covered with SAMCO grey (Samco Agricultural Manufacturing Ltd.) photodegradable mulch film. Such mulch films protect the plugs from desiccation (Zhou et al., 2009), late frost, pests (e.g. rabbits) and can accelerate the plant development especially in climates with lower spring temperatures (Farrell & Gilliland, 2011). Preliminary UK trials since 2014 showed that thereby commercial yield levels (>10 Mg DM ha^{-1} $year^{-1}$) can be reached at the end of the second growing season, a year faster than without film (Ashman et al., 2018). Many sites did not use this technology because of concerns over stimulating weed competition, heat stressing and leaving undecomposed plastic residues in soil (Ashman et al., 2022). Where SAMCO mulch films were not used, limited irrigation was applied several times during the first month following planting to prevent propagule desiccation (Table S3). During the planting period, the ZAG site was affected by a significant drought period, after which a rainy period prevented the timely application of herbicides and encouraged the rapid growth of weeds. Detailed information on the management of the field trials is found in Table S3.

2.5 | Phenotyping and harvest protocol

Figure 2 shows the position of plants in each plot and the fixed measurement areas within the plots of the five marked plants used for regular phenotyping of growth and the harvest area of minimum 6.6 m^2.

In the autumn following planting, the counting of plant was used to assess the survival rates at the end of the first growing season before the first winter. To assess hybrid yield potential with complete leaf canopies at the target planting densities, lost plants within the measurement areas of each plot were filled by transplanting form border plants from the plot edges into the gap positions in spring/early summer 2019.

The five marked plants in each plot were used to assess a range of yield traits in autumn following the growing season, and in spring around the time of harvest (Figure 2). In a few instances, these were reduced to three plants to expedite phenotyping. Since these plant-level measurements were used to create a trait means at plot level keeping the four replicate blocks ($n = 4$). Traits phenotyped in autumn at the end of each successive growing season before winter frosts included height (cm), visual scores of canopy greenness (where a score of 0 is fully brown and 9 is fully green (data in Table S4)) and lodging (where 0 is fully standing and a score of 9 indicates all shoots are lying flat to the ground). Plant basal diameter, and a count of 'big shoots per plant' (at least 60% of the tallest shoot) used to calculate shoots per square meter accounting for planting density where either conducted in autumn or spring after winter ripening.

Plant height was measured to the plant canopy in the first and second years and to the last ligule height the third year at all sites except SCH (which used canopy height throughout), the difference between these methods is dwarfed by inter-site and year variation. If plants had lodged, the measurements were taken by measuring the length of a canopy-forming shoot to the ligule of the last fully expanded leaf.

The flowering scores were recorded on a 0–4 visual scale. A flowering score of 1 indicates the most advanced shoot on a marked plant has produced a 'flag leaf', while a score of 2 is recorded on the date when panicles have emerged by at least 1 cm (more than stage 50; Tejera & Heaton, 2017). A score of 3 indicates that more than 50% of the big shoots have emerged panicles which coincides with anthers shedding pollen and stage 4 indicates that flowering is complete because the anthers can no longer be seen, and if the plants have had a partner in pollen range the panicles are likely to contain seed.

The harvest and border area (red and grey box in Figure 2) were positioned the same for all plots at each site. Keeping the boulder area around the plot ensured the harvest area was always away from the edge of the plot. In springtime (February to March) following each growth year (2018/2019 = 1st year, 2019/2020 = 2nd year and 2020/2021 = 3rd year), quadrat harvests were made with a cutting height of 10 cm above the ground surface with a hedge trimmer. The bundle of shoots was weighed to 50 g accuracy before a subsample of approximately five stems per was randomly selected. The fresh weight of the subsample was determined either immediately in the field (with a wind protected balance) or bagged in plastic and weighed within several hours. The subsamples were dried until constant weight at 105°C and reweighed. The dry weight of the harvest quadrat (Q_D) was calculated from the quadrat fresh weight (Q_F) minus the moisture content of the subsample ($S_{F/D}$).

$$Q_D = Q_F - \left(Q_F \times \frac{(S_F - S_D)}{S_F} \right).$$

2.6 | Statistical analysis

Phenotyping data were collected by android app and uploaded to a customised plant breeding database (Physis Data Ltd), using phenotyping names outlined in Clifton-Brown, Schwarz, et al. (2019). All analysis was done in R (R Core Team, 2015). The yield, moisture content and phenotyping data were analysed with a robust three-way ANOVA of Year, site and hybrid; for all the analysis of establishment, a two-way ANOVA of site and hybrid was used. To disaggregate within-site hybrid variation, a one-way ANOVA on hybrid with a year site combination was run with a Bonferroni adjustment, allowing a Tukey's HSD to identify which hybrids were different in that year.

3 | RESULTS

3.1 | Environmental conditions

Over the three suited years, OLI had the coldest spring temperatures (−2.1 to 3.9°C average monthly temperature) while PAC1 was the warmest (5.4 to 9.5°C). Summer average maximum daily temperatures in PAC1 reached 29°C (Table S2). SCH followed by CHV had most average wind per day (12.5 and 17.7 km h^{-1}). The temperature at TWS was very varied leading to several frosts and warmer weeks each year. The ZAG and PAC1 sites had a large difference between the maximum and minimum temperatures. Overall, the coldest temperature was measured at OLI (−18.2°C) and the warmest at CHV (41.6°C); however, the warmest on average was PAC1.

TWS had 50% higher precipitation than the next wettest site ZAG (891 mm), while the driest site was CHV (537 mm). Soil water deficits showed short droughts occurred in CHV location in the second and third years. The monthly water balance for the CHV site showed the long period of deficits in the middle of the growing season contributing to the low-yield potential at this site. Both PAC sites had several soil water deficits in all 3 years but in the second and third years this was quite localised to the middle of summer.

Over the 3 years, of the trial PAC2 is most affected by soil water support without it the trial would have experienced large droughts, with or without ground water there were high soil water deficits in ZAG, PAC1, CHV and SCH. The lowest rainfall fell in CHV and Scheme (537 and 473 mm on average per year, respectively). While all sites had some frosts, OLI had the longest lowest winter temperatures (Magenau, Clifton-Brown, Parry, et al., 2022). The OLI and PAC2 sites had inter-year soil water deficits although the method to calculate then was annual, assuming zero on January 1.

3.2 | Crop establishment

Establishment success of plug plants raised from seed and rhizomes split from overwintering dormant plants is known to comprise several phases: namely survival following planting in the first month (transplanting success), survival following the first whole year (when the plant re-emerges from the rhizome) and continues growth and maturation in the second year and subsequent years (overwintering success).

The transplant success rates for plug and rhizome planted *Miscanthus* hybrids varied widely between the different sites and hybrids. CHV, SCH and ZAG had the lowest establishment (52%–60%) while the highest establishment was in TWS, OLI and PAC1 (88%–86%). The transplanting success of the standard clonal $M \times g$ (GRC 9) from rhizomes was significantly lower than the plug planted hybrids. The plug planted *sac*×*sin* hybrids survived significantly better than the *sin*×*sin* hybrids especially in the lower latitude sites. GRC 15 which has a more creeping rhizome than standard $M \times g$ survived significantly better but was worse than the plug planted hybrids (Figure 3).

Plant losses were primarily due to drought before effective roots had grown into the soil. Even sites that were watered at planting due to dry soil (e.g. OLI), had losses due to one of the most prolonged droughts in recent European history in 2018. Weeds also threaten the first year *Miscanthus*. As some weed species have the potential to 'shade out' the slow-growing *Miscanthus* plug plants in the first few months after planting. The choice and timing of herbicides and/or mechanical weed control depended on the weed burden/species at each site and the herbicides used locally to control these weeds (details in Table S4).

Overwinter plant losses observed the following May on re-emergence occurred primarily in TWS in the *sac*×*sin* hybrids which, following several days of unseasonably warm weather in late February 2019 (Figure 4), stimulated very early shoot emergence. Many of these unhardened plants succumbed to three successive spring frost events (Figure 4), which killed many plants, notably in GRC 14. In OLI shoots that emerged early in spring 2019 were also killed by a single severe late frost, but all plants re-sprouted new shoots from the rhizomes and few plants died. In contrast, at the start of the second year (Figure 4), OLI was affected by a strong frost which affected *sin*×*sin* hybrids more than the *sac*×*sin* that emerged later (Magenau, Clifton-Brown, Parry, et al., 2022). Low over winter temperatures at OLI did

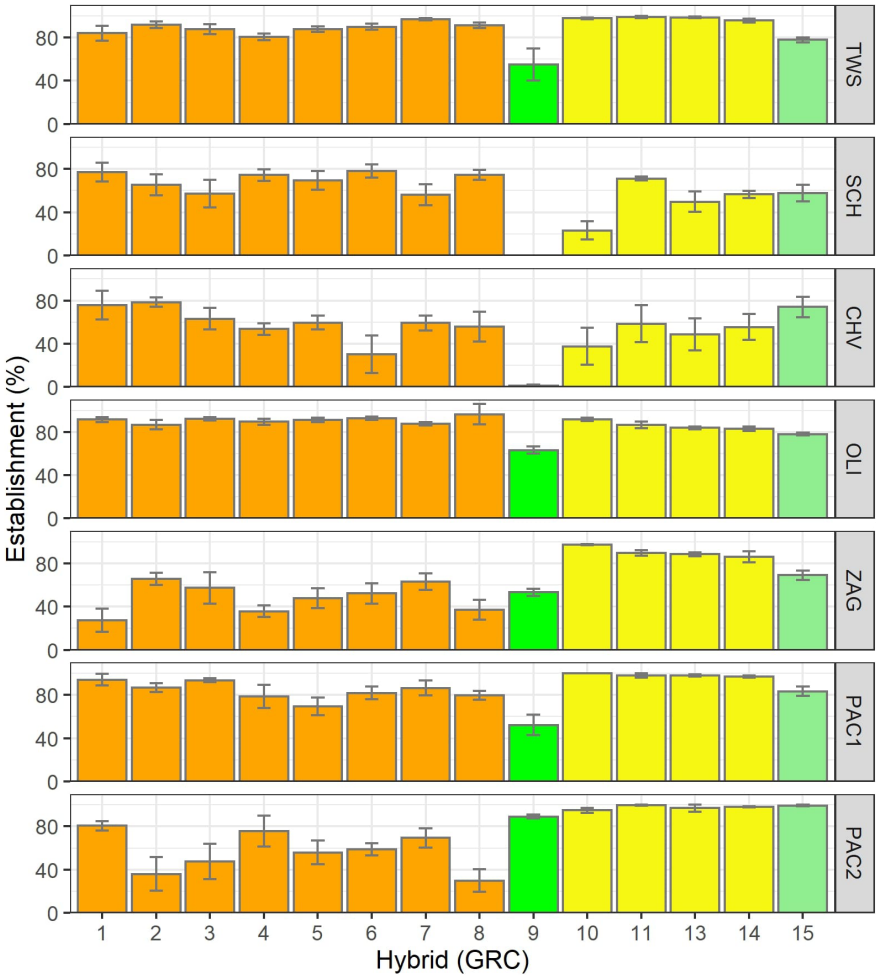

FIGURE 3 Plant establishment over the whole first year (2018), across seven sites in latitude order (see Figure 1). *Miscanthus sin×sin* hybrids (GRC 1–8, orange), *sac×sin* (GRC 10–11, 13–15, yellow) hybrids, and rhizome planted *M×g* (GRC 9, green) and GRC 15 (*sac×sin*, light green), $n = 4$, error bar = ±1 standard error.

not lead to overwinter losses due the isolation function of the snow layer.

3.3 | Composite yield traits

In the first year, the average number of shoots (m^{-2}) over all sites was 39 in *sin×sin*, 12 in *M×g* and 27 in *sac×sin* hybrids. In the third-year *sin×sin* and *M×g* had increased more than threefold (136 and 37 shoots m^{-2}, respectively) while *sac×sin* hybrids less than doubled (51 shoots m^{-2}). However, once planting density removed this discrepancy is reduced though the number of stems per plant was still higher for *sin×sin* (45 shoots per plant) than for the *sac×sin* hybrids (34 shoots per plants) in the third year. As the *sin×sin* hybrids are planted at twice the density, these normally have higher numbers of stems per square meter than *sac×sin* hybrids, this difference is most pronounced by the third year (136 in *sin×sin* to 52 in *sac×sin* by the third year).

The number of shoots contributing to the canopy was significantly impacted by site ($p < 0.001$), hybrid ($p < 0.001$) and growth year ($p < 0.001$) (Figure 5). Specifically, GRC 11 had significantly higher shoot counts than the other *sac×sin* hybrids and GRC 4 had significantly higher shoot counts than all other *sin×sin* and *sac×sin* hybrids. Also, GRC 4 has significantly ($p < 0.05$) more stems than any hybrid in TWS in the first year and PAC1 in the second year (Figure 5).

ANOVA showed autumn peak plant heights differed significantly between sites ($p < 0.001$), years ($p < 0.001$) and hybrids ($p < 0.001$). It was notable that the average across sites and years for the tallest hybrids GRC 15 and GRC 14 were 1.9 and 1.7 m, respectively, which was significantly taller than all the other hybrids ($p < 0.05$). GRC 15 and GRC 14 grew to 2.6 and 2.4 m, respectively, in the third year. The *sac×sin* hybrids were taller than *sin×sin* across most sites and years while the height of the *M×g* varied widely between sites (Figure 6). The average mature heights of the *sin×sin* and *sac×sin* hybrids were 1.8 and 2.2 m, respectively, in the third year. At ZAG and SCH, the rhizome-based hybrids were significantly taller in the third year than *sin×sin*.

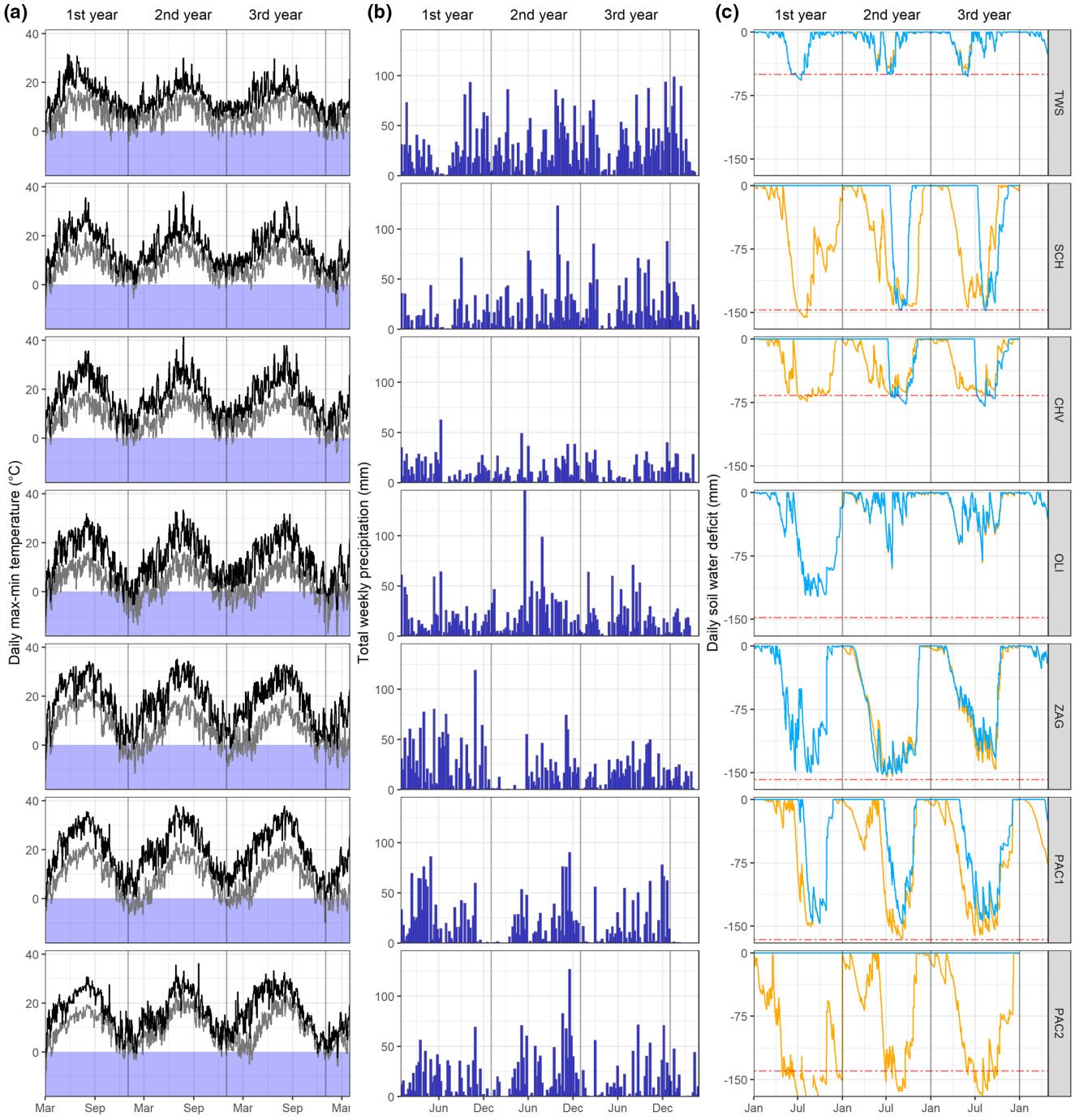

FIGURE 4 Graphs of (a) daily average air temperature at 2 m (maximum in black and minimum in grey, blue highlights sub-zero temperature). (b) Total precipitation per week. (c) Calculated daily soil water deficit with plant available water with (blue) and without (yellow) soil water support. The red line shows plant available water. Over seven sites in latitude order (see Figure 1).

In the first year, the average hybrid heights across all seven sites were 0.7 for $M \times g$ and $sin \times sin$ and 1 m for $sac \times sin$. On average, the $sac \times sin$ hybrids increased with age; by 86% to an average height of 1.8 m in year two and just 26% (2.2 m) in year three. While $sin \times sin$ hybrids average height increased by 91% (1.3 m) and 45% (1.8 m) from the first to second and second to third years, respectively.

3.4 | Flowering

Flowering scores in October showed that almost all $sin \times sin$ hybrids flowered in all locations in all years, but that $sac \times sin$ hybrids only flowered in warmer sites at lower latitudes (Figure 7). Of the $sac \times sin$ hybrids, GRC 15 reached flowering scores of 2 as far north as the SCH site each year. The second year had less flowering in the

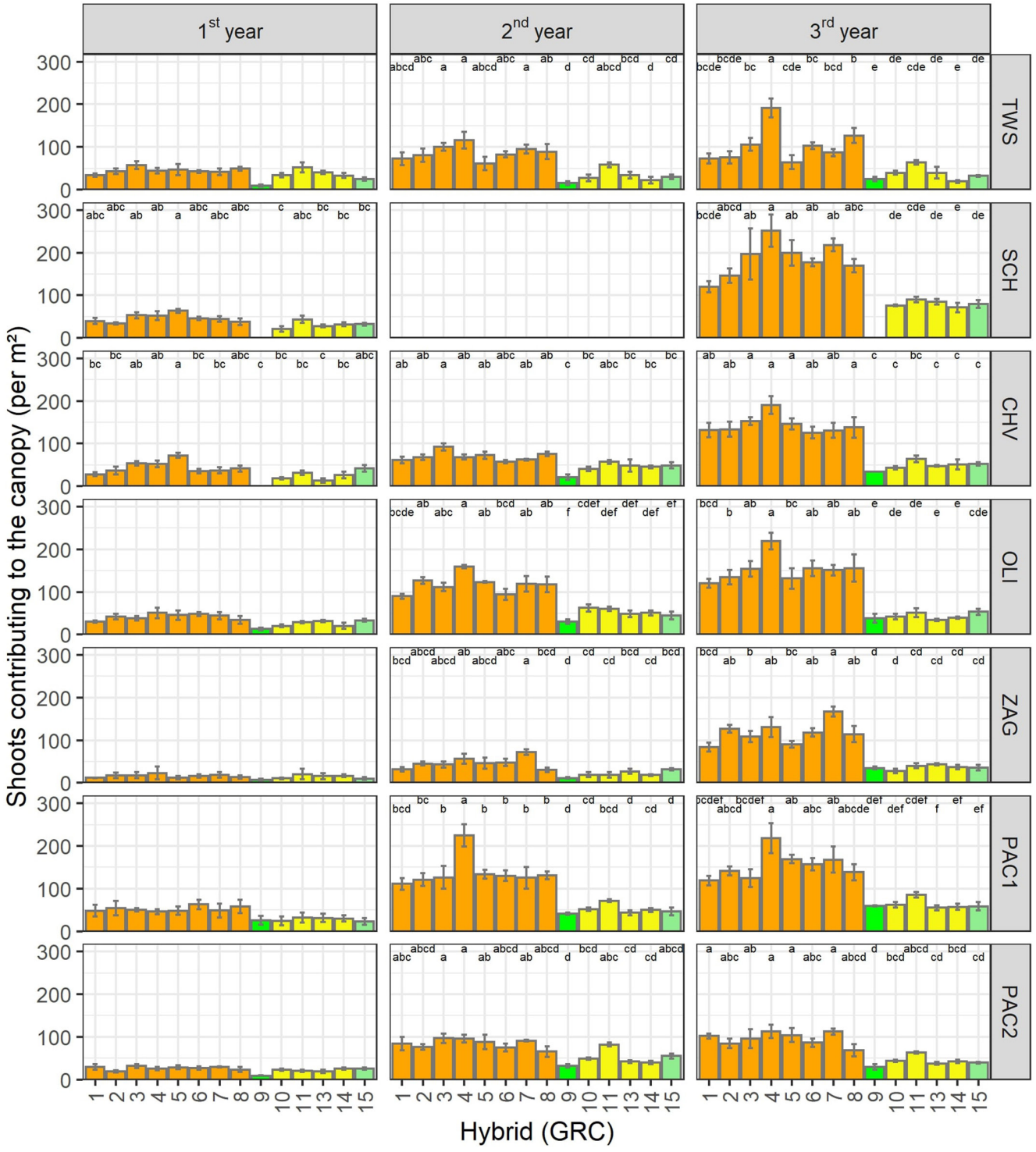

FIGURE 5 Shoots per m^2 contributing to the canopy measured on the senesced crop in the first (2018), second (2019) and third (2020) growing years, across seven sites in latitude order (see Figure 1). *Sin*×*sin* hybrids (GRC 1–8, orange), *sac*×*sin* hybrids (GRC 10, 11, 13, 14, yellow), and rhizome planted *M*×*g* (GRC 9, green) and GRC 15 (light green), $n = 4$, error bar = ±1 standard error. Different 'a, b, c' letters indicate that hybrid means within a site and year are significantly different (at $p < 0.05$ with a Tukey test). Stem numbers were not counted in SCH in the second year.

sac×*sin* hybrids than the first or third years this trend is less noticeable in the *sin*×*sin* which increase flowering with maturity in all sites except PAC that dips in 2019 (second year).

3.5 | Lodging

There was no lodging recorded in the first year while the plants were establishing and averaged less than 0.9 m

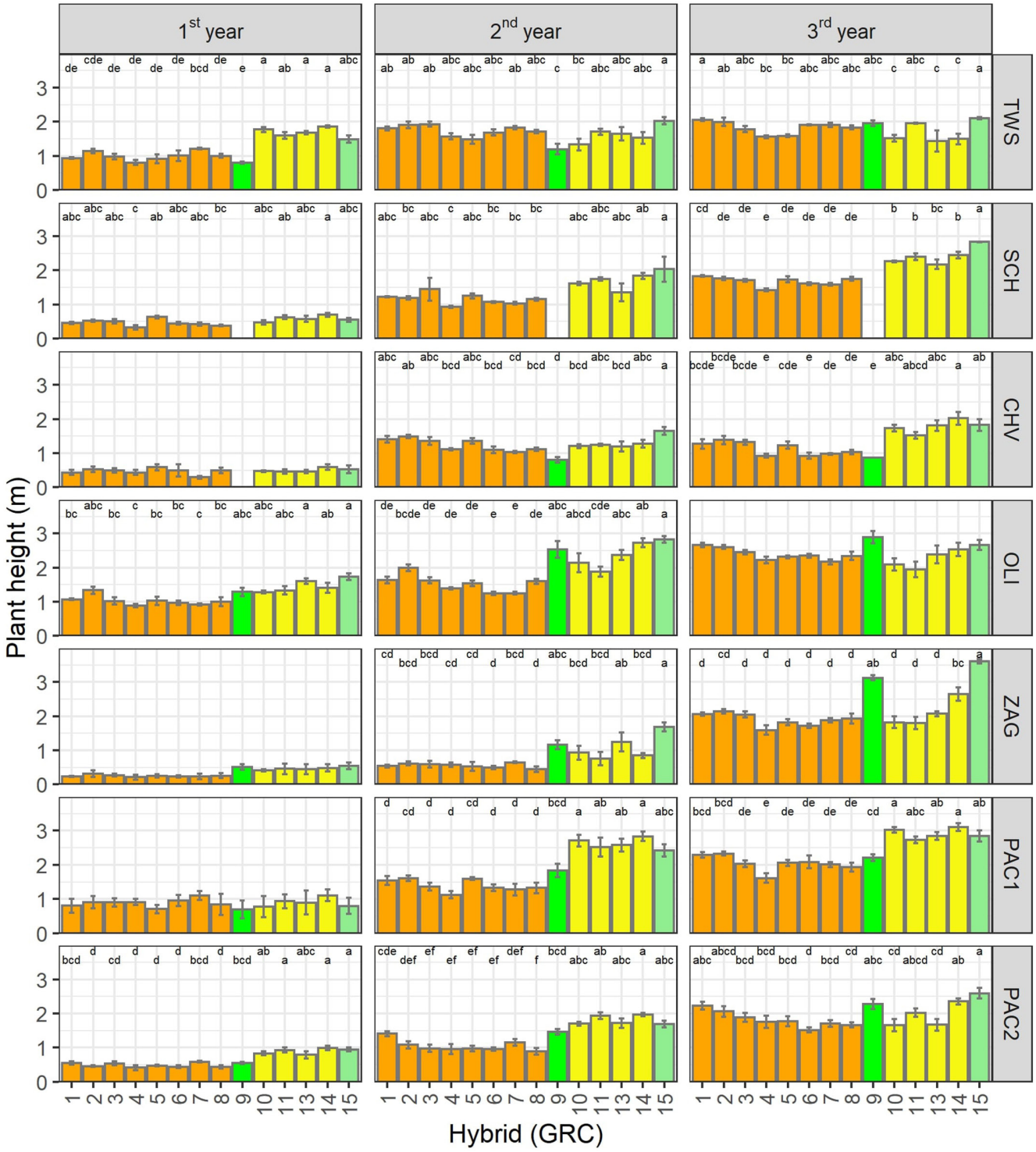

FIGURE 6 Plant heights in autumn measured on the senesced crop in the first (2018), second (2019) and third (2020) growing years, across seven sites in latitude order (see Figure 1). *Sin*×*sin* hybrids (GRC 1–8, orange), *sac*×*sin* (GRC 10–11, 13–15, yellow) hybrids, and rhizome planted *M*×*g* (GRC 9, green) and TV1/GRC 15 (light green), $n = 4$, error bar = ±1 standard error. Different 'a, b, c' letters indicate that hybrid means within a site and year are significantly different (at $p < 0.05$ with a Tukey test).

tall. In the second, when the average height was 1.5 m, some lodging was observed, but no consistent data were recorded until the third year. During the third growing season, strong interplant competition occurred with canopy closure and lodging was observed in several sites by late August 2020. At this point, a lodging score was employed to capture the hybrid-specific patterns within and across sites at peak height in autumn of the third year, there was no lodging in the PAC or SCH sites, only a small amount in ZAG but in the more northerly sites OLI and

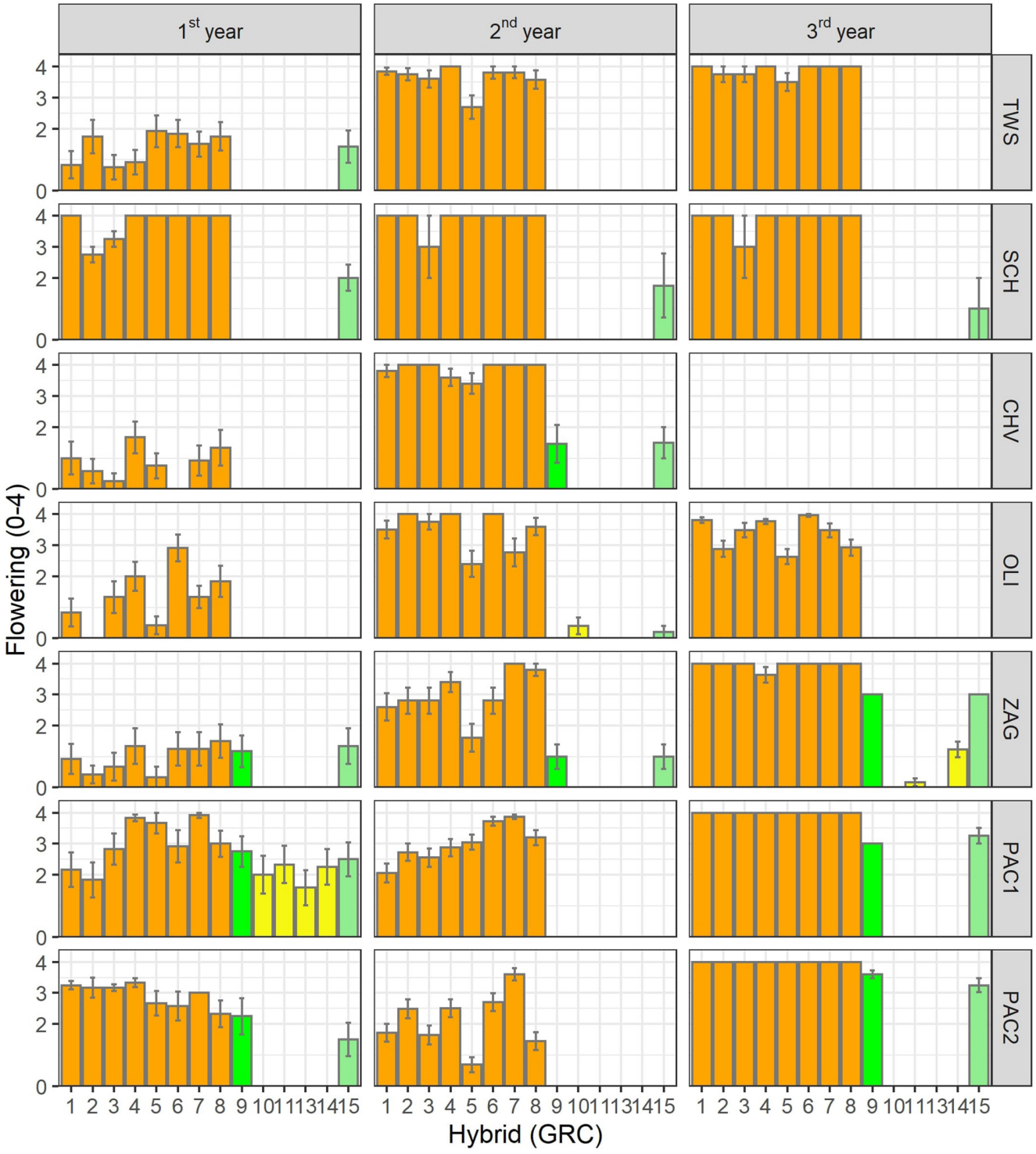

FIGURE 7 Mean flowering scores 0 not flowered to 4 fully flowered measured in autumn derived from weekly observations between late September and early November in the first (2018), second (2019) and third (2020) growing years. Over seven sites in latitude order (see Figure 1). $n = 4$, error bar = ±1 standard error. No data for year 3 in CHV.

TWS significant lodging occurred in some of the *sin*×*sin* hybrids and the tallest hybrid GRC 15 (Table 2). However, during the winter (November) of the third growing season, a 'wet' snowfall before the leaves had detached froze onto the shoots collapsed the stems of all hybrids in both PAC sites, and the crop remained under the snow for several months. When the snow melted, the shoots sprang up to about 30 cm, this allowed the stems to dry out before harvest in the more exposed and lower precipitation site (PAC2).

3.6 | Plant basal diameter

Plant basal diameter averaged 23 cm in the *sin* × *sin* hybrids, 29 cm in the *sac* × *sin* hybrids and 46 and 47 cm in the rhizome-based hybrids *M* × *g* and TV1/GRC 15 (Table 3). *M* × *g* and GRC 15 were the largest stands in all sites apart from TWS. In SCH, ZAG and PAC1, these two most spreading hybrids had twice the median basal diameter than the other hybrids. There was little difference in the basal diameter across all hybrids in TWS by the third year. In PAC sites, the basal diameter increased in all the *sac* × *sin* hybrids over the 3 years.

3.7 | Yields

PAC1 produced the highest yields over the two establishment years, with averages of 4.3 and 11 Mg DM ha^{-1} (Figure 8). The lowest yielding site was CHV, producing an average of 4.2 Mg DM ha^{-1}. Across all sites, the average yield in the second year was 6.6 Mg DM ha^{-1} higher than in the first year by 5 Mg DM ha^{-1}, this increased to 9.8 Mg DM ha^{-1} in the third year. The average third yield of GRC 10–15 hybrids was higher than the GRC 1–8 *sin* × *sin* (11 and 9.1 Mg DM ha^{-1}, respectively) standard *M* × *g* (GRC9) was 9.7 Mg DM ha^{-1}. Overall, the highest yielding *Miscanthus* hybrid was GRC 14 but the yields still had a wide range 2.5–19.7 Mg DM ha^{-1} in the second year and 4.9–21 Mg DM ha^{-1} in the third year. The second and third highest yielding *Miscanthus* hybrids (GRC 11 and 15) were more consistent across sites in the third year with higher minimum yields of 5.6 and 6.2 Mg DM ha^{-1}, respectively.

Figure 8 shows over all 3 years that the lower altitude site at PAC1 produced significantly higher average yields (9.7 Mg DM ha^{-1}) than all other sites ($p < 0.05$), followed by OLI (7.6 Mg DM ha^{-1}) which was also significantly different from other sites ($p < 0.05$). CHV produced the lowest average yields (4.2 Mg DM ha^{-1}) but was not significantly different to SCH and ZAG.

As SCH did not have any *M* × *g* in the harvest quadrats in the second and third years, an example quadrat yield for *M* × *g* was taken from a neighbouring field. These plants were of the same age, this field trials calculated (from a 5-plant harvest) third growing season yield would have been 18.8 Mg DM ha^{-1} (21.2% moisture content) with complete establishment. This yield was most equivalent to GRC 15 yields following gap correction (from 13.5 to 17.4 Mg DM ha^{-1}). This yield (sown in Figure 8 as red dot) provides an indication of the potential of *M* × *g* at SCH with good establishment.

After good establishment in TWS and OLI in the first year, these sites had the highest yields among the northerly sites (1.8 and 1.6 Mg DM ha^{-1}, respectively); however, by the second year, TWS yield dropped in comparison to OLI after repeated second year spring frosts (4.9 and 8.9 Mg DM ha^{-1}, respectively). At these higher latitude and cooler sites in OLI and TWS in the third year, the *sin* × *sin* hybrids yielded more than the *sac* × *sin* hybrids, though *M* × *g* yielded most (11.1, 9.4, and 11.4 Mg DM ha^{-1}, respectively). TWS produced the lowest yields in the seed-based *sac* × sin hybrids following the third growing season (7.3 Mg DM ha^{-1}), here the *sin* × *sin* hybrids planted at the high planting densities (3 plants m^2) produced the higher yields (9.4 Mg DM ha^{-1}).

Figure 9 shows the only strong correlation between yield of different years was between the second and third

Hybrid	TWS	SCH	OLI	ZAG	PAC1	PAC2
GRC 1	0.6 ± 0.3	0	2 ± 0.2	3.5 ± 0.6	0	0
GRC 2	6.9 ± 0.3	0	5.5 ± 0.5	4.5 ± 0.3	0	0
GRC 3	3.2 ± 0.5	0	1.5 ± 0.3	3 ± 0.7	0	0
GRC 4	2.1 ± 0.6	0	5 ± 0.6	3 ± 0.6	0	0
GRC 5	4.9 ± 0.5	0	5 ± 0.4	3.5 ± 0.6	0	0
GRC 6	1.3 ± 0.4	0	4.5 ± 0.6	0	0	0
GRC 7	0.8 ± 0.4	0	5 ± 0.6	0	0	0
GRC 8	4.9 ± 0.6	0	2.1 ± 0.3	3 ± 0.7	0	0
GRC 9	0		1 ± 0.3	0	0	0
GRC 10	0	0	0	0	0	0
GRC 11	1.3 ± 0.4	0	0.8 ± 0.4	0	0	0
GRC 13	0.7 ± 0.3	0	0.2 ± 0.1	0	0	0
GRC 14	0	0	0.4 ± 0.1	1.5 ± 0.6	0	0
GRC 15	1.7 ± 0.2	0	3.8 ± 0.5	4.5 ± 0.2	0	0

TABLE 2 Lodging at the end of the third growing season on (October 2020) across six sites (sites are in order of descending latitude (see Figure 1), $n = 4$). This used a 0–9 scale from no lodging to fully lodged, averages and standard error of the four replicate blocks given. Data not collected in the CHV site.

TABLE 3 Plant basal diameter in cm for each hybrid at each site (in latitude order see Figure 1), after the third growing season with standard error of the four replicate blocks. Data not collected in the CHV site.

Hybrid/site	TWS	SCH	OLI	ZAG	PAC1	PAC2
GRC 1	22.3 ± 1.7	15.3 ± 5.3	25.2 ± 1.7	17.4 ± 1.8	26.6 ± 1.7	24.4 ± 2.6
GRC 2	22.4 ± 2.1	25.7 ± 2.1	24.9 ± 1.3	17.6 ± 1.0	27.1 ± 1.5	27.6 ± 0.6
GRC 3	23.7 ± 0.6	25.6 ± 6.2	26.0 ± 1.0	18.4 ± 1.3	32.0 ± 1.8	24.3 ± 2.2
GRC 4	24.3 ± 1.6	21.9 ± 2.9	27.6 ± 2.5	18.6 ± 1.9	27.2 ± 1.2	23.8 ± 1.7
GRC 5	17.9 ± 0.4	21.4 ± 2.3	24.0 ± 1.6	16.0 ± 1.5	27.5 ± 0.2	22.1 ± 1.9
GRC 6	24.0 ± 0.7	19.4 ± 4.6	26.4 ± 1.9	17.2 ± 0.7	26.4 ± 0.4	23.6 ± 1.7
GRC 7	21.2 ± 1.9	21.1 ± 3.9	24.6 ± 1.8	19.8 ± 0.5	27.0 ± 1.3	24.1 ± 0.6
GRC 8	23.2 ± 1.1	27.1 ± 2.4	24.2 ± 0.2	17.5 ± 1.7	26.0 ± 1.2	24.5 ± 1.3
GRC 9	28.0 ± 1.2		39.8 ± 4.4	55.9 ± 2.8	58.0 ± 6.1	50.5 ± 3.2
GRC 10	29.1 ± 2.2	27.7 ± 7.8	31.7 ± 3.0	26.1 ± 2.9	37.5 ± 1.2	32.2 ± 0.5
GRC 11	26.0 ± 0.4	26.7 ± 4.3	25.4 ± 1.3	24.5 ± 2.1	32.9 ± 0.8	32.9 ± 0.3
GRC 13	26.7 ± 0.7	21.0 ± 7.6	28.8 ± 2.3	23.1 ± 1.9	32.5 ± 2.2	29.1 ± 2.8
GRC 14	23.3 ± 1.7	28.0 ± 2.2	32.6 ± 1.8	28.9 ± 3.0	44.4 ± 2.6	36.2 ± 1.5
GRC 15	30.7 ± 2.0	49.2 ± 9.5	47.5 ± 3.6	51.9 ± 5.1	64.8 ± 4.2	39.4 ± 2.2

years ($R^2 = 0.48$) with first to third the weakest ($R^2 = 0.19$), with first to second between ($R^2 = 0.32$). The site with the closest correlation was PAC2 across 1st:2nd, 1st:3rd and 2nd:3rd years ($R^2 = 0.65, 0.47, 0.71$, respectively). The only site with a stronger correlation in any year was PAC1's second-year to third-year correlation ($R^2 = 0.75$).

In the first- to second-year comparison, the *sac*×*sin* hybrids had a better overall correlation, with GRC 14 having an R^2 of 0.55; however, the clonal types GRC 9 and GRC 15 had unusually poor correlations (both $R^2 = 0.04$). This pattern held but was less clear in the first- to third-year comparison. The second to third year also had the same pattern of GRC 14 strongly linked to the prior year ($R^2 = 0.69$) and GRC 15 with the weakest correlation ($R^2 = 0.2$), *M*×*g* (GRC 9) had a much better link when using second-year data ($R^2 = 0.55$).

The sites at which hybrids gave the highest and lowest yields were diverse with nine hybrids performing best in at least one site and 1 year (Table 4). In the mature third year, six out of the seven sites had a unique best performing hybrid with only GRC 15 being the highest at two sites.

Table 4 shows a clear different pattern for locations north and south of OLI. The more southerly sites have an improved performance from *sac*×*sin* hybrids, while the northerly sites have a more mixed rank order.

The top-ranked plant in the first year is never the top raked plant by the third year. GRC 9 (*M*×*g*) is most often the lowest yielding hybrid; however, poor establishment at SCH and CHV hinders *M*×*g*. Also, as *M*×*g* is slow at maturing, in both PAC sites and TWS *M*×*g* improves in ranking with year and in the OLI site *M*×*g* is lowest in the first year but highest yielding by the third year (Table 4).

3.8 | Moisture content

Figure 10 shows complex dynamics for moisture contents at spring harvests in three consecutive years across all seven field sites. Overall sites in the first year the *sin*×*sin* hybrids had a similar moisture content to the *sac*×*sin* hybrids averaging 40%. In second and third years, the *sac*×*sin* hybrids (42% and 40%, respectively) were on average wetter than *sin*×*sin* hybrids (23% in the second and 28% in the third years).

At the northerly location TWS, the moisture content of the *sin*×*sin* hybrids in the first and second years were similar at 35%, but in the third year these fell dramatically to 20%. In CHV, first year moisture contents were also high (60%) but fell to 20% in the second and third years. These moisture content reductions with crop age were not reflected in the southerly locations PAC1 and ZAG, where moisture contents rose in the third year.

4 | DISCUSSION

In contrast to the earlier *Miscanthus* projects GRACE focused on seed-based hybrids (12 out of 14) where past European *Miscanthus* projects had zero (EMI: European *Miscanthus* improvement (Lewandowski et al., 2003)) and only 2 out of 15 (OPTIMISC: Optimizing Miscanthus Biomass Production (Lewandowski et al., 2016)). The GRACE plots were double the size of the trials in EMI and OPTIMISC and instead of three replicated randomised blocks per sites, there were four to help statistically distinguish between hybrids and their associated agronomies. In the EMI and OPTIMISC projects, a

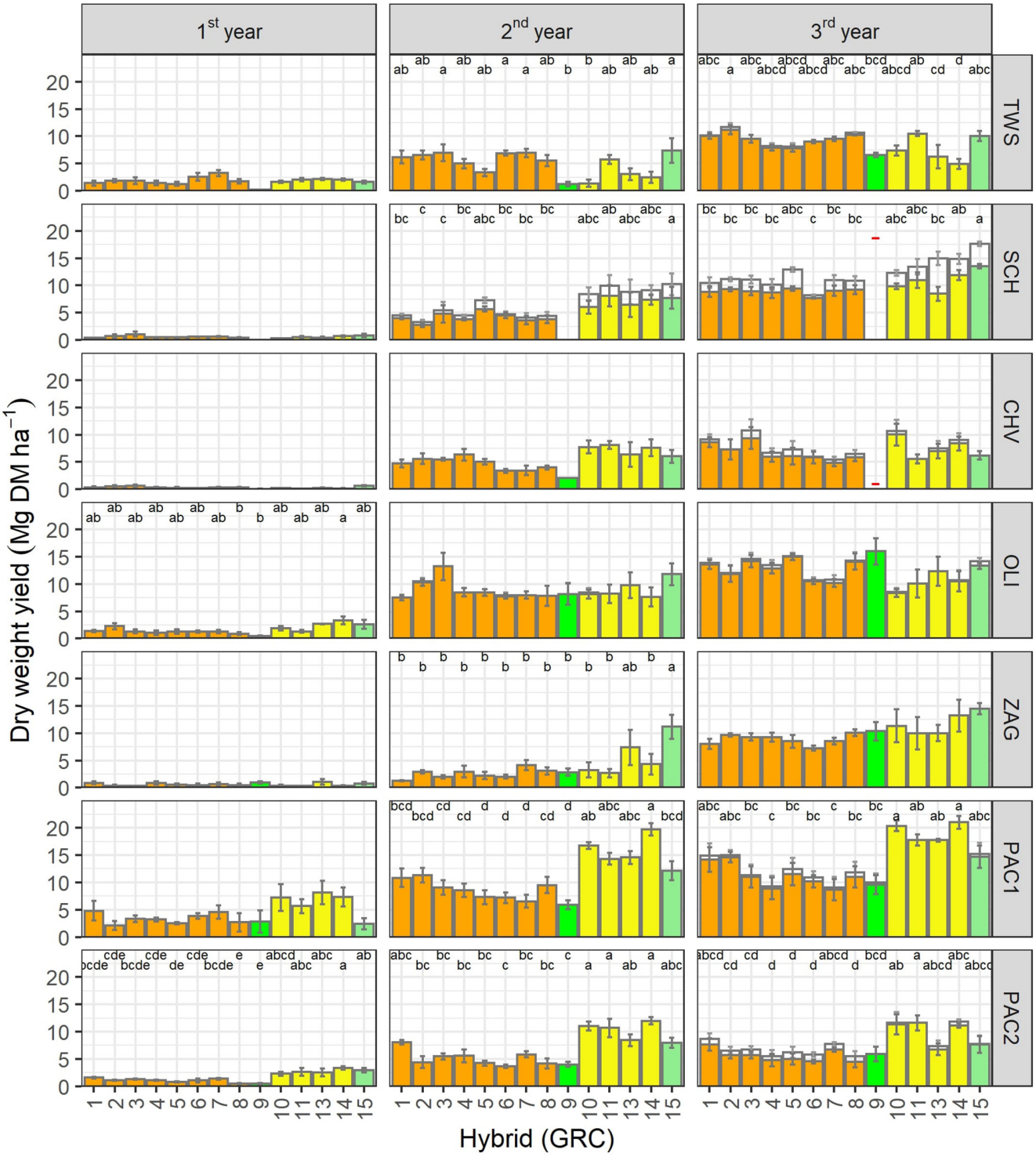

FIGURE 8 Harvested yields following the first (2019), second (2020) and third (2021) years, a gap corrected yield has been added for the second and third years as a clear bar. *Sin*×*sin* hybrids (GRC 1–8, orange), *sac*×*sin* (GRC 10–15, yellow) hybrids, and rhizome planted *M*×*g* (GRC 9, green) and GRC 15/TV1 (light green). Red dots are shown where missing data had comparable yields information from nearby experiments. Error bar = standard error, $n = 4$. Different 'a, b, c' letters indicate that hybrid means within a site and year are significantly different (at $p < 0.05$ with a Tukey's test). Seven sites in latitude order (see Figure 1).

standard historically proven planting density of two plants m^{-2} was used for all hybrids. In GRACE, planting densities were adjusted to match the compact morphology of the *sin*×*sin* (30,000 plants ha^{-1}) and the likely creeping morphology of the *sac*×*sin* (15,000 plants ha^{-1}) to be around the commercial density of *M*×*g* (15–17,500 plants ha^{-1}) (Caslin et al., 2011; DEFRA, 2007; Terravesta, 2017, p. 6).

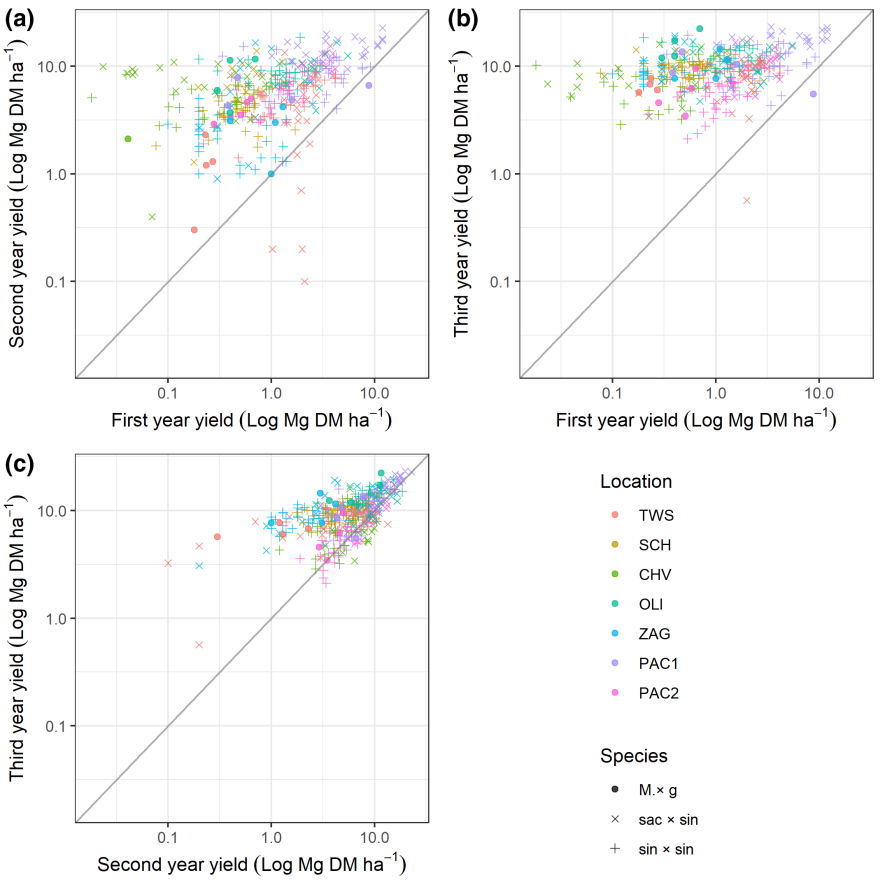

FIGURE 9 A comparison of yields between (a) 1st and 2nd, (b) 1st and 3rd, and (c) 2nd and 3rd years. Yields are shown per replicate and have been logged to distribute the points. Linear relationships show consistently of yield. The grey line shows the 1:1 yield relationship where yields were the same both years. Over seven sites (see Figure 1).

The distribution of the field sites in EMI ranged from Sweden to Portugal, and in OPTIMISC from Moscow to Adana (Turkey). The range of locations in GRACE from Wales to Croatia is more restricted, but nonetheless covers the climatic ranges for most of Western Europe. In contrast to these prior studies, all GRACE trials were planted on difficult marginal land sites. Despite this, yields in the GRACE trials were in general comparable to EMI and OPTIMISC trials in the third year (GRACE = 9.8 Mg DM ha^{-1}. OPTIMISC = 9.9 Mg DM ha^{-1}, EMI = 11.4 Mg DM ha^{-1} (excluding losses)). The future advantage of the seeded hybrids is its scalability when the technology (hybrid selection and agronomy) is mature enough to overcome plant losses. In addition, diverse hybrid variants have the advantage of resilience matching to local end-user needs through trialling and modelling. A diversity of hybrids is also needed to protect against climate extremes and potential disease threats. Diversity could be a disadvantage if biomass of a certain standard is traded widely, as for the commodity food crops such as wheat.

Reducing the *sac*×*sin* density is likely to have extended the length of the yield building phase in cooler locations were the rhizomes spread more slowly. For *M. sinensis*, higher density planting has been shown to decrease the time to reach full yields but not the maximin yield (Ouattara et al., 2020). In GRACE, higher density *sin*×*sin* did not produce notably higher yields in the first year but did keep pace with the *sac*×*sin* over the 3 years. While using double density was very useful for commercially relevant comparisons between *sin*×*sin* and *sac*×*sin* hybrids, as only one density within each type was used comparisons can only be made in the context of the cultivation system. A future trial with the same *sin*×*sin* and *sac*×*sin* hybrids planted on both 1.5 and 3 plants m^{-2} would allow understanding the effect of density and hybrid on long-term yield dynamics. The third-year basal diameter measurements show how *M*×*g* and GRC 15 grew out to fill more space in as they matured, this implies that these plants in particular will make use of the lower density planting to expand and add yield while more compact plants (e.g. GRC 13) at the lower density will not.

Stem counts per plant for *sin*×*sin* hybrids were higher than for *sac*×*sin* hybrids over the first 3 years. With the planting density of three plants m^{-2} for *sin*×*sin* shoot numbers m^{-2} ranged from 64 to 251 across all sites in the third year. The *sac*×*sin* hybrids planted at a planting density of 1.5 plants m^{-2} had more than 30% fewer shoots m^{-2} (19–90 across all sites) than the *sin*×*sin*. Third-year dry weights per stem for the *sac*×*sin* hybrids were 227% higher than for *sin*×*sin*. This was reflected in both greater plant height and stem diameters. Thus, shoot height had a much stronger effect on yield than stem count even in

TABLE 4 Mean plot ranking for all hybrids in each year with across all sites last, split into the first (2019) second (2020) and third (2021) year harvests. Sites by site code, and hybrids identified by GRC number; colours added to identify the *sin*×*sin* in orange, the *sac*×*sin* in hybrids in yellow, and the rhizome-based *M*×*g* and TV1 in dark and light green, respectively.

Site	Year	Highest yield								Lowest yield					
TWS	1st	7	6	13	11	14	2	3	8	10	15	4	1	5	9
	2nd	15	3	7	6	2	1	11	8	4	5	13	14	10	9
	3rd	2	11	8	15	1	3	7	6	4	5	10	9	13	14
SCH	1st	3	15	14	2	7	6	11	5	4	13	8	1	10	
	2nd	11	15	14	13	10	5	3	6	1	8	4	7	2	
	3rd	15	14	11	10	5	2	8	7	3	1	4	13	6	
CHV	1st	3	15	2	7	4	1	8	5	6	13	10	14	11	9
	2nd	11	10	14	13	4	15	2	3	5	1	8	7	6	9
	3rd	10	3	1	14	2	13	15	5	4	6	8	11	7	9
OLI	1st	14	13	15	2	10	1	7	11	5	3	6	4	8	9
	2nd	3	15	2	13	4	5	11	9	10	7	8	6	14	1
	3rd	9	5	3	8	1	15	4	13	2	14	6	7	11	10
ZAG	1st	13	9	1	4	15	7	5	8	6	2	11	10	14	3
	2nd	15	13	14	7	10	8	4	2	9	11	5	6	3	1
	3rd	15	14	10	9	8	13	11	2	4	3	7	5	1	6
PAC1	1st	13	14	10	11	1	7	6	3	4	9	8	5	15	2
	2nd	14	10	13	11	15	2	1	8	3	4	5	6	7	9
	3rd	14	10	11	13	15	2	1	5	8	3	6	9	4	7
PAC2	1st	14	15	11	13	10	1	7	3	4	2	6	5	8	9
	2nd	14	10	11	13	1	15	7	4	3	2	5	8	9	6
	3rd	11	10	14	1	15	13	7	9	3	2	5	4	6	8
All	1st	13	14	10	11	7	15	1	6	3	2	4	5	8	9
	2nd	15	14	11	13	10	3	2	1	4	7	8	5	6	9
	3rd	14	15	10	11	1	2	13	3	9	8	5	4	7	6

the *sin*×*sin* hybrids. For example, shorter *sin*×*sin* hybrids in SCH than at OLI still yielded less despite higher stem counts. This has been identified before (Davey, Robson, et al., 2017; Kalinina et al., 2017), though some studies have shown a strong link between stem number and yield (Gauder et al., 2012; Robson et al., 2013). However, the shorter and thinner stemmed *sin*×*sin* hybrids have several advantages at spring harvest because they often ripen faster resulting in lower moisture content (Robson et al., 2011) and potentially require less powerful machinery to harvest either directly into chip with a forage harvester or into bales through the two-step process of mowing followed by baling (Hastings et al., 2017). While stem number contributes less than height to yield, it seems clear that the *sin*×*sin* hybrids require the density to achieve the yield.

Moisture contents below 20% of the harvested biomass are needed to reduce inefficiencies associated with the transportation of water (Khanna et al., 2008; Valentine et al., 2012), to prevent microbial spoilage during transport and storage (Huisman & Kortleve, 1994) and increase the efficiency of end use (dti, 2007; Lewandowski & Heinz, 2003). Biomass exploitation for bioproducts and biopower both benefit from lower moisture contents (Clifton-Brown & Lewandowski, 2002; Iqbal et al., 2017). For climates with mild wet winters where the more productive *sac*×*sin* hybrids are grown harvest methods are being developed to reduce the moisture before the crop is transported from the field, such as the common technique in wetter climates of cutting to a swath (Hastings et al., 2017; Lewandowski et al., 2000). This moisture content is very affected by leaf to stem ratio at harvest, with leaf contributing more moisture (Magenau, Clifton-Brown, Awty-Carroll, et al., 2022).

Moisture content and greenness show a reversal where more northerly sites produce a drier crop in later years with little senescence in the first year, while southerly sites produce a dryer crop in the first year when the plants are smaller, then moisture content increases in subsequent years. However, the moisture content is still

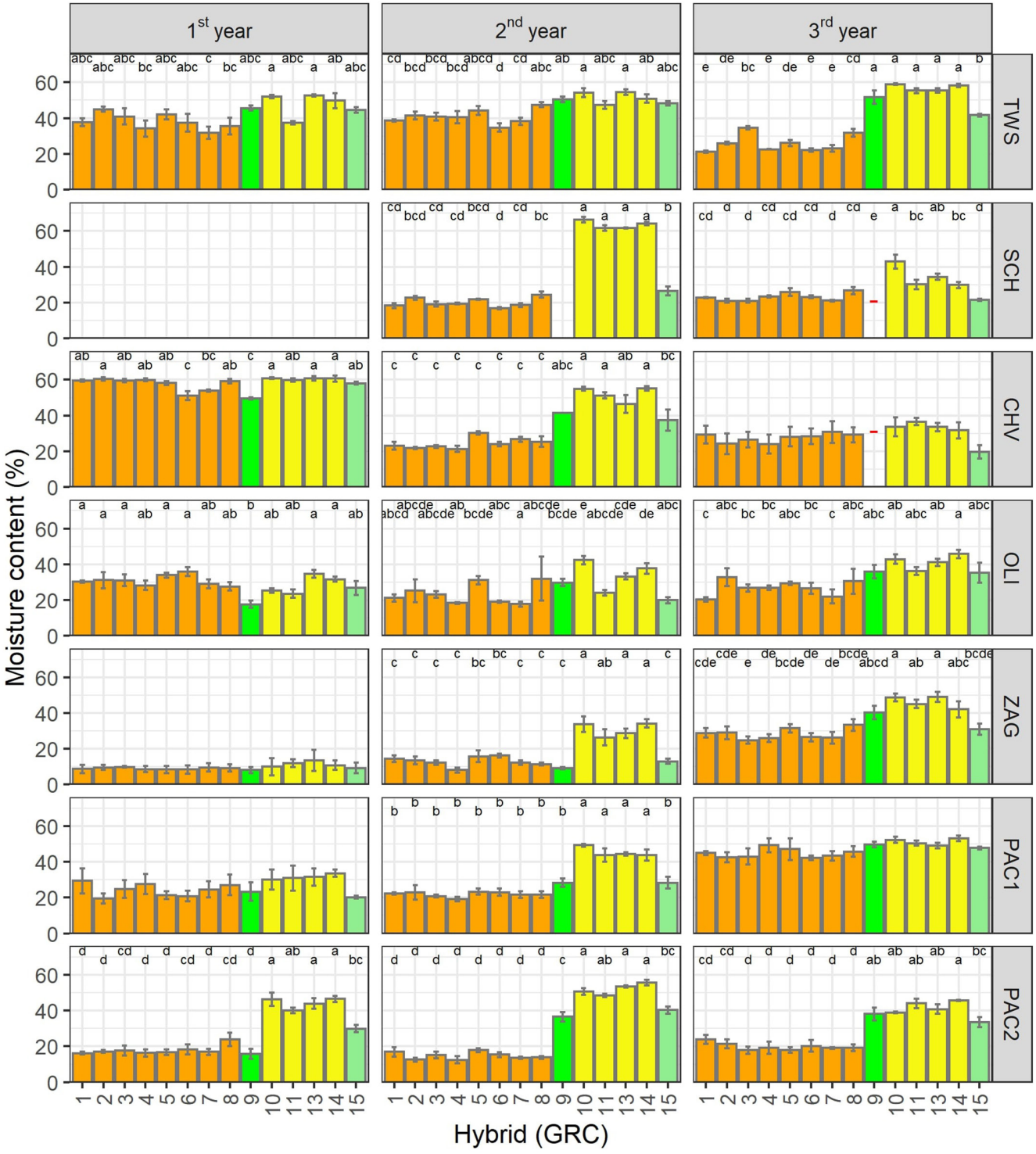

FIGURE 10 Trial harvest moisture content in spring harvests in the first (2019), second (2020) and third (2021) years: *Sin*×*sin* (GRC 1–8, orange), *sac*×*sin* (GRC 10, 11, 13, 14, yellow) hybrids, and rhizome planted *M*×*g* (GRC 9, green) and TV1/GRC 15 (light green). Error bar = standard error, $n = 4$. Different 'a, b, c' letters indicate that hybrid means within a site and year are significantly different (at $p < 0.05$ with a Tukey test). Over seven sites in latitude order (see Figure 1). Moisture content was not calculated for SCH in the first year as the whole quadrat was dried rather than subsampled. For *M*×*g* in the third year at SCH and CHV a comparable quadrat moisture content was calculated from a neighbouring trial of *M*×*g* of the same age this was found to have a moisture content of 21.2% shown as a red dot.

lower in the southerly latitudes by the third year. This is unlikely to be due to drought as most southerly sites did not experience severe water deficits and any atmospheric drying would have been more at colder northerly sites. Therefore, this is probably due to a maturity effect as the plant matures faster in southerly latitudes. The overall

higher moisture content in the *sac*×*sin* hybrids than the *sin*×*sin* hybrids can largely be explained by the physiological differences in flowering. But intercomparison is difficult as all *sin*×*sin* hybrids flower and none of the *sac*×*sin* hybrids flower at all sites. The dry mass yield of the *sac*×*sin* hybrids where they did flower did not result in a smaller yield; however, this depends on growth and flowering time as a plant can grow faster to achieve flowering (Dong et al., 2019). Also, strong water defects in early autumn could affect the flowering (Jensen et al., 2011; Nunn et al., 2017) and senescence (Clifton-Brown et al., 2002).

4.1 | Optimal hybrid systems for the different regions of Europe

Based purely on yield (Mg DM ha^{-1}) collected over 3 years, the *sin*×*sin* hybrids are better adapted to regions with cool summers and mild winter such as the Wales (UK) than the *sac*×*sin* hybrids. *sin*×*sin* hybrids flower too early in warm locations but in cool climates these are better adapted than the *sac*×*sin* hybrids. For central Europe, both *sin*×*sin* and *sac*×*sin* hybrids may be appropriate depending on local climate and end uses. In the southern European climate, the *sac*×*sin* hybrids have much higher yield potential and achieve moisture contents that are low enough for both direct chip and swath-to-bale harvest approaches. Northern latitude yields were not predictive of southern latitude yields in *sin*×*sin* hybrids this has previously been observed in the United States (Clark et al., 2019).

As well as yield, the *sin*×*sin* with thinner stems were easier to harvest with a longer harvest window, on account of earlier flowering and ripening. With softer stems, they also probably require less expensive harvest machinery than the *sac*×*sin* hybrids whose stems are thicker and stronger.

The data are short term, still at most sites the crop may have plateaued in yield by the third year due to the marginal conditions; however, it may take one to two more years to reach a ceiling yield (Caslin et al., 2011; Miguez et al., 2008; Ouattara et al., 2020). Therefore, longer-term studies are needed to assess the interactions between hybrid type×planting density×environment (climate and soil) to provide the information for crop production sustainability criteria.

The GRACE results across locations expose each of these first-generation seeded hybrids strengths and weaknesses. To give two examples: First, (GRC 10) achieved ~10 Mg DM ha^{-1} at the CHV site in the third year. CHV which includes industrially damaged land, polluted and with poor water retention properties. Combined with low rainfalls and high evaporations associated with the Paris basin, this represented very poor conditions for *Miscanthus* establishment. Second, while a fast yield building rate is vital for the economic viability of seed-based *Miscanthus* (Hastings et al., 2017; O'Loughlin et al., 2017), the top yielding *sac*×*sin* hybrid GRC 14 emerges early and ripens late to maximise the growing season (Davey, Jones, et al., 2017; Davey, Robson, et al., 2017), but moisture levels are too high for direct chip harvests and plants are susceptible to damage from late frosts (Farrell et al., 2006; Magenau, Clifton-Brown, Parry, et al., 2022). This demonstrated the breeding trade-off between long growing seasons to maximise yield and that effect on biomass quality.

4.2 | Improvements needed in agronomy and breeding

These GRACE trials have produced early-stage evaluation for a set of 13 novel hybrids compared to the control genotype *M*×*g*. As seen in earlier breeding trials where *M*×*g* was planted as a control, the relative performance of *M*×*g* compared to the newer hybrids strengthens over time. This slow phenotypic maturation of *M*×*g* makes it difficult for breeders to select the best hybrids quickly and is used to justify the development of genomic selection approaches in *Miscanthus* as well as other perennial biomass crops such as switchgrass, poplar and willow (Clifton-Brown, Harfouche, et al., 2019). The first-year yields were also not indicative of second or third year yields, this demonstrates an important limitation in *Miscanthus* breeding where selections of outstanding plants are made in the second year (Clifton-Brown et al., 2017; Clifton-Brown, Schwarz, et al., 2019) in an attempt to shorten the selection for plot-scale evaluation and breeding cycles.

The crop establishment reported here is part of an ongoing programme to develop seed-based *Miscanthus* technologies. In an earlier paper, establishment experiments with direct sown seeds showed that low temperatures and-or low moisture contents made establishment highly unreliable (Ashman et al., 2018). Consequently, direct sowing was not considered to establish these large GRACE experiments in so many different locations. Instead, an indirect seed-to-plug and plug-to-field planting system was developed. Propagation for these GRACE project field trials pushed new boundaries for upscaling plug production and the logistics for getting plugs at the right time to the field locations. Establishment of both *M*×*g* and the seeded hybrids was lower than expected (61%–74%), this was partly due to droughts in 2018. The effect of drought was increased as due to transportation issues there was a second shipment of plugs pushing planting later. These

delays did show what works for large-scale plug transport that has not been done before in *Miscanthus*. The maize mulch film is known to be beneficial for the successful establishment of *Miscanthus* (O'Loughlin et al., 2017), it protects delicate plugs from damage by grazing and late frosts and increases growth rates by increasing the soil temperature, improving hydraulic contact and retaining moisture (Easson & Fearnehough, 2000; van der Werf, 1993; Zhou et al., 2009). This film was effective in reducing expected transplanting losses facing into a 3-month drought from May to July in 2018. This mid-growing season drought in 2018 occurred all over northern Europe resulting in widespread crop failures in Maize and large reductions in cereal yields (Brás et al., 2021). But at locations with warmer conditions and no grazing the mulch confers less benefit, and disadvantages include stimulation of weeds and overheating (Clifton-Brown, Schwarz, et al., 2019).

4.3 | Limitations

There is always a difficulty harmonising the methods across all sites in large multi-location trials run from different institutions. Each site had small local differences in agronomy due to location, weather conditions and marginality. Due to this, planting and harvest dates varied within a month between sites. Across multiple sites measurements in particular greenness and flowering can be affected by phenotyper that could only be consistent within a site.

Some *M*×*g* rhizome had a suspected infection with Fusarium which made comparisons to it difficult at CHV and impossible at SCH. Gaps created by plants losses during early establishment over the first year, due to desiccation following transplanting and frost damage during spring emergence, were filled to ensure the target planting densities. Repairs to plots in this way were justified for research into yield potential of the mature crop but shows clearly plug-based planting systems for seeded hybrids require further detailed work to minimise the risk of plant losses. Newer projects are already underway to explore interacting factors to produce more resilient planting systems (Wu et al., 2021).

4.4 | Conclusions

These results in contrast with earlier studies show a specificity of hybrid to the environmental conditions. Across the seven sites in the third year, there were six different best yielding hybrids. *Sac*×*sin* hybrids are best adapted to locations with long warm growing seasons because flowering occurs late or not at all before the onset of winter. *Sin*×*sin* hybrids flower too early in warm locations but in cool climates these are better adapted than the *sac*×*sin* hybrids. Therefore, there is a large potential for crop improvement drawing on the natural diversity within and between the *M. sacchariflorus* and *M. sinensis*. As the European *Miscanthus* breeding programmes mature, recurrent selection within species groups in different environments is expected to select positive alleles for yield and quality traits. These also enhance the potential for heterosis like effects in interspecies crosses. The *sac*×*sin* hybrids in these trials are only one step away from the wild accessions, data from trials such as those being performed in GRACE can be used to fine tune the selection of parents with the potential for better performance in different environments.

These results show that further developments are needed to optimise the seeded hybrids, breeding for establishment and resilience remains vital for a perennial crop but has a trade-off with yield and quality. Also, these data suggest breeding within hybrid types for height over stem number to achieve yield and making the selections in the second or third years. For now, clonal hybrids will continue to play an important role in developing biomass supply from *Miscanthus*. To reach the European planting areas approaching 13 Mha (Don et al., 2012), sustained long-term breeding work on seeded hybrids is essential to the missions of producing novel hybrids with all steps covered from seed production to final yield and composition.

AUTHOR CONTRIBUTIONS

Danny Awty-Carroll: manuscript and data analysis. **John Clifton-Brown, Elena Magenau & Chris Ashman**: manuscript editing. **Annick Hoogendam, Chris Ashman, Enrico Martani, Mislav Kontek, Philip van der Pluijm, Michele Croci & Elena Magenau**: site management, data collection and assistance with data analysis. **Emmanuel de Maupeou, Jon McCalmont, Gert-Jan Petrie, Chris Davey, Kasper van der Cruijsen, Vanja Jurišić, Stefano Amaducci, Isabelle Lamy, Annick Hoogendam, Oene Dolstra & Anita Shepherd**: specific parts of the experimental setup instrumentation and data frameworks. **Oene Dolstra, Mohamad Al Hassan & Jason Kam**: for the plant materials used. **John Clifton-Brown, Andrea Ferrarini, Isabelle Lamy, Luisa M. Trindade, & Andreas Kiesel**: study design.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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