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Ashman, C, Awty-Carroll, D, Mos, M, Kam, J, Guerrini, S, Calder, S and Clifton-Brown, J (2023) Developing Miscanthus seed plug establishment protocols with mulch film for commercial upscaling. GCB Bioenergy, 15 (6). pp. 746-764. ISSN 1757-1693

Received: 20 December 2022 | Accepted: 18 January 2023
DOI: 10.1111/gcbb.13044

GCB-BIOENERGY
BIOPRODUCTS FOR A SUSTAINABLE BIOECONOMY
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RESEARCH ARTICLE

Developing *Miscanthus* seed plug establishment protocols with mulch film for commercial upscaling

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Abstract
High-yielding crops with C_4 photosynthesis arising in tropical climates are being bred for, and increasingly grown in, temperate climates. *Miscanthus*, a C_4 from Eastern Asia is a leading perennial biomass crop, but commercial deployment is limited by low temperatures in Northern Europe, low clonal multiplication rates and slow establishment rates requiring up to 4 years to reach mature yields. While new seeded hybrids have multiplication rates >2000, direct field sown seed has proven impractical. Protocols for safe establishment of seeded hybrids require that seedlings are raised in the glasshouse in compost filled modules (also known as 'plugs') which are transplanted into the field in springtime. To protect seedlings from damage from late frosts, drought and grazing and to increase temperature stimulating growth rates, plug plants were covered with oxo-degradable plastic mulch film designed for maize. At two sites in the UK, this mulch film significantly reduced plant losses at transplanting and overwintering, increased stem heights and shoot counts, and reduced the time to mature yield from 4 to 3 years ($p < 0.01$). However, the breakdown products of oxo-degradable mulch films contribute to microplastics in the soil. Therefore, further mulch film experiments were conducted with bio-derived plastics which are bio-degradable in soil at extruded thicknesses of 10, 18 and 30 microns. The 10 micron film combined sufficient strength for machine laying and worked as well as oxo-degradable film on de-risking establishment. Halving the mulch film widths covering 1 row rather than 2 reduced the amount of plastic by 25%. Commercial plug-to-field protocols are built on results from the plot experiments and field-scale plantings over multiple years and locations and are ready for future upscaling of biomass production from seed-based *Miscanthus* hybrids.

KEYWORDS
agronomics, biodegradable mulch film, circular economy, improving yield, increasing establishment speed, *Miscanthus*, mulch film, perennial biomass crop, reducing establishment cost, upscaling

Chris Ashman and Danny Awty-Carroll contributed equally to this work.

1 | INTRODUCTION

Perennial biomass crops (PBCs) such as *Miscanthus* and Willow have the potential to be an alternative to fossil fuels and could help to mitigate climate change (Creutzig et al., 2015). Furthermore, energy from biomass crops could be carbon negative if combined with carbon capture and storage (BECCS) (Fajardy & Mac Dowell, 2017). In addition, PBCs have the potential to offset fossil fuels through providing feed stocks for a range of industrial end uses such chemical production, building materials and transport fuels (Götz et al., 2022; Lask et al., 2021; Witzleben, 2022). While PBCs have a high potential to substitute fossil fuels for a range uses the planted areas of such crops remains low in the UK with just over 8000 ha of *Miscanthus* being grown in 2020 a rise from 6900 ha in 2015 (DEFRA, 2021; von Hellfeld et al., 2022; Witzel & Finger, 2016). The UK's Committee for Climate Change reported that to reach the government's net zero target by 2050, that 23,000 ha of PBCs need to be planted every year between 2020 and 2050 (CCC, 2020). Rapid upscaling and deployment of PBCs are needed to reach the UK governments net zero goals.

Miscanthus a C_4 grass is a high-yielding PBC with low moisture content at harvest that has been shown to have high potential as a dedicated biomass crop in Europe (Clifton-Brown et al., 2004; Hastings et al., 2009; Heaton et al., 2004). *Miscanthus × giganteus* (*Mxg*) a sterile triploid has been widely tested in Europe (Heaton et al., 2010; Lewandowski et al., 2000) and once established, continues to be highly productive for 15–20 years (Lesur et al., 2013; Lewandowski et al., 2003). Due to the sterility of *Mxg*, it cannot be established from seed (Linde-Laursen, 1993) and therefore must be established clonally.

Mxg is normally established by planting rhizome pieces directly harvested from a 'mother field'. Propagation of *Miscanthus* using this method has a multiplication factor (mf) of between 10 and 30 (Hastings et al., 2017; Xue et al., 2015) and therefore the current upscaling potential is restricted. Alternative clonal propagation methods such as plantlets produced indirectly from rhizome or micro-propagation have a higher mf of ×30 and ×960, respectively. However, production costs of these methods are >€4200 ha^{-1} and >€6000 ha^{-1}, respectively (Xue et al., 2015) which also limit scalability. An alternative to establishing *Miscanthus* clonally is to establish crops from seed. Seed-based *Miscanthus* hybrids offer significantly greater upscaling potential than rhizome with a mf of >1500 and could enable faster introduction of new cultivars (Clifton-Brown et al., 2017). Furthermore, introduction of new hybrids may aid development of new end uses as specific traits could be selected through breeding.

High establishment rates are required for high yields in all crops, perennial or annual (Atkinson et al., 2007; Finch-Savage & Bassel, 2015). Poor establishment leads to gaps in the crop, slow canopy closure and allows addition competition from weeds species consequently increasing the need for herbicide application. Furthermore, poor establishment of perennial crops will not only limit yield in the establishment year but will limit yield for the productive lifespan of the crop. Gaps caused by poor establishment in *MxG* have been shown to reduce gross margins by more than 50% and increase the payback time from initial investment (Dauber et al., 2015; Zimmermann et al., 2014). In addition, poorly established PBCs with lower yields are likely to sequester less carbon in the soil reducing the potential for climate change mitigation.

Novel seed-based *Miscanthus* hybrids have been developed by multiple breeding programs (Clifton-Brown et al., 2019) as an alternative to clonal production. However, trials of seed establishment from direct sowing of *M. sinensis Anders* have had limited success and require high quantities of seed (Ashman et al., 2018; Christian et al., 2005). Seedlings from seed sown into compost filled modules 'plug plants' raised in the glasshouse before transplanting into the field offers an alternative to direct sowing whilst still utilizing seed-based hybrids and is widely used for a range of horticultural crops (Cantliffe, 2008; Orzolek, 1991).

Such plug planting systems allow massive improvements in *Miscanthus* crop scalability. However, there may be additional risks to planting *Miscanthus* plug plants compared to rhizome planting. Rhizomes act similarly to seeds, initiating growth once conditions are favourable. Unlike propagation with dormant rhizomes, plug plants are actively growing when they are planted and thus need immediate access moisture and nutrients to avoid transplanting losses resulting in gaps.

Mulch films increase soil temperature and reduce moisture losses thus improving growing conditions (Easson & Fearnehough, 2000; O'Loughlin et al., 2017). Crop establishment, yield and quality of thermophilic crops such as Maize have been shown to be improved in cooler climates such as northern Europe or Canada through the use of mulch films (Farrell & Gilliland, 2011; Kwabiah, 2005). Mulch films can be produced in a range of materials, these include polyethylene films that need to be manually removed or degradable films such as oxo-degradable film and biodegradable mulch films (Kasirajan & Ngouajio, 2012). Oxo-degradable plastics comprise of standard plastics with additives to aid break up by UV and heat. As such, they do not fully biodegrade and could leave microplastics in soils (Abdelmoez et al., 2021; Aisbl & Deconinck, 2013), the use of Oxo-degradable plastics has been banned in the EU Directive 2019/904 (European Parliament, 2019).

While the benefits of mulch films for some crops are clear allowing the geographical range of many crops to be expanded these benefits must be weighed up against the possible environmental cost. In Europe over 720 Gg of plastic materials are used in agriculture and only 24% of this (mainly greenhouse films and drip tubes) is recycled, while 29% is landfilled. Furthermore, 34 Gg per year of plastic materials is still burned in the field and it has been estimated that in Southern Europe about 950 kha are contaminated with plastics (Eunomia, 2021). Poor recovery of non-degradable mulch films can lead to plastic remaining in the soil (Liu et al., 2014) and degradation of plastic mulch films can lead to micro plastics in soil (Li et al., 2022; Yang et al., 2022). Currently, it is not standard practice to use mulch film to aid establishment of *Miscanthus* from rhizome in northern Europe due to the additional cost. However, trials have shown that use of mulch film increased rhizome viability, reduced time to viable yield and increased yield (Olave et al., 2017; O'Loughlin et al., 2017).

We hypothesized that mulch film would improve establishment and overwintering success of seed-based *Miscanthus* hybrids established from plug plants and accelerate growth in the first year. This paper evaluates (1) the efficacy of conventional oxo-degradable mulch film on improving the establishment, overwinter success and yield of seed-based *Miscanthus* plug plants, (2) the impact of novel bio-degradable in soil (BIS) mulch films on establishment and overwintering of seed-based *Miscanthus* plug plants and (3) the utilization of commercial machinery such as plug planters and mulch film layers to establish commercial-scale *Miscanthus* plots. We report progressive learnings from multiple field trials and produce planting protocols for seed-based *Miscanthus* plug plants utilizing existing on farm equipment and experience.

2 | MATERIALS AND METHODS

Below are the details of four field trials established to assess the impact of mulch film on the establishment, first year growth and subsequent overwinter survival of novel seed-based *Miscanthus* hybrids established from seed-based plug plants.

2.1 | Plant production

Winter sown plantlets were produced by Bell Brothers Nursery Ltd. They were grown in glass houses, in module trays, and hardened off prior to planting. The plug plants were sown in early January, plantlets were grown in the nursery for approx. 4.5 months. Day length was extended to 12 h through supplemental light at either end of the day. The glasshouse was heated to between 20 and 25°C during the day and 15 and 20°C during the night. Prior to planting, the plantlets were moved to a cooler unlit glasshouse to harden off. At planting, plantlets had 2–3 stems, approximately 30 cm long. Several novel seed-based hybrids *M. sacchariflorus* × *M. sinensis* interspecific hybrids bead by Aberystwyth University (IBERS) were used, these were given the names GNT 1, GNT 2 and GNT 4; also precommercial hybrid GRC 10 used in European field trials (Awty-Carroll et al., 2023). Commercial UK sourced *Mxg* (named GRC 9) rhizome was supplied by Terravesta (Lincoln, UK). Summer sown GRC 14 plantlets were produced by Fountain Plants Ltd. The plug plants were sown in early August 2020 in glasshouses under ambient conditions and grown under glass until planting in late April 2021 following standard Brassica production methods. Heat was only provided over winter to ensure glasshouse temperatures did not drop below 2°C and no additional light was provided. Plantlets were grown in 216 re-useable module trays widely used within the vegetable production sector.

2.2 | Land preparation and location

Three field trials, in trials 1a and 1b, mulch film and planting density trials and (2) a comparison of oxo-degradable mulch film and BIS mulch films were established in 2014 and 2019, respectively. Furthermore, in trial 3, an unreplicated commercial scale trial (9.4 ha) was planted in 2020 to assess the commercial feasibility of establishing *Miscanthus* from seed-based plug plants with mulch film. Trial 1a was established in Aberystwyth, West Wales at the Institute of Biology and Environmental Research Studies (IBERS), whereas trials 1b, 2 and 3 were established at Hackthorn, Lincolnshire in collaboration with Terravesta Ltd. All sites were representative of land available for the establishment of *Miscanthus* crops within the trial site regions. Previous land use had been long-term grassland at Aberystwyth (ABR) and arable rotation at Hackthorn (HCK). Soil type differed between the sites from sandy loam (SL) at and Aberystwyth and Silty Clay (SC) at Hackthorn.

Prior to establishing the trials, each sites trial area was sprayed with a glyphosate-based herbicide to remove both broadleaf and grass weeds, and the trial areas were ploughed and cultivated to provide a fine tilth for planting. After planting, Stomp® Aqua (BASF Agricultural Solutions UK) was applied at 1.5 lt ha^{-1} prior to covering each plot with mulch film. Weeds were controlled in year

1 by mechanical removal (strimming between plants) from mid-July (once the film had degraded).

2.3 | Plant growth measurements

In the first year, establishment rates were measured just after the mulch film had degraded (approx. 6–8 weeks after planting). Overwintering success was measured at the beginning of the second growing season after the last frost (late may). Phenotypic measurements of stem count and stem height were measured for all trials. Stem count included all stems above 50% of canopy height, stem height was measured to the top ligule of a representative stem. Measurements were collected from plants within the centre of the plot, edge rows were dismissed for phenotypic measurement to remove any bias due to edge effects.

Yield was assessed in years 2 and 3 for trial 1, year 1 harvest was not collected as it is not commercial practice to harvest *Miscanthus* crops after the first year's growth. Quadrats were taken from the centre of each plot and weighed in field using a tripod and hanging balance. Subsamples from each plot were chipped and oven-dried at 105°C to assess moisture content and calculate dry matter yield. Yield measurements were not collected on trials 2 and 3.

3 | TRIAL-SPECIFIC METHODS

3.1 | Mulch film

Five mulch films were tested in the trials described below. They include Samco 'grey' oxo-degradable mulch film and BIS mulch films with a range in width and thickness (Table 1). BIS mulch films used in these trials are fully BIS in accordance with EU standard EN 17033 (REF). The bioplastic for the BIS mulch films was developed by Novamont (under the trade name Mater-Bi) and is plant derived. All BIS mulch film were extruded by Samco, mulch film thickness and width.

3.2 | Trial 1a and 1b assessing the impact of Samco grey mulch film and planting density on the establishment and overwinter survival of 4 novel seed-based hybrids and GRC 9 planted at two densities

In 2014, two field trials were planted, one at IBERS (Aberystwyth University) and one at Hackthorn Lincolnshire, (Terravesta Ltd.) to measure the effect of mulch film and planting density on seedling establishment, first year growth and overwintering success of four seed-based hybrids established from plug plants and *Mxg* (GRC 9) established from rhizome. Samco 'grey' a 1.3 m wide 7 μm thick oxo-degradable mulch film was tested each row of mulch film covered two rows of plants. Four interspecific seed-based hybrids GNT 1, GNT 2, GNT 4 and GRC 10 and a rhizome established hybrid GRC 9 were selected for these trials The selection criteria of the four seed-based hybrids were (1) high seed set and (2) high germination success rates. The plantlets were grown by Bell Brothers Ltd. in 126 module trays; the modules tray cells were 2 by 2 cm, and 4 cm deep with a volume of 25 cm^3. Module tray selection criteria were as follows: (1) It was expected to provide enough rooting volume for *Miscanthus* plug over the nursery period, (2) It was compatible with existing commercial-scale seed sowing equipment at the nursery and (3) It allowed plugs to produced densely in the nursery optimizing nursery space. A standard commercially available peat-based compost was selected for its moisture and nutrient-holding properties.

Trial 1a was planted at Aberystwyth, the trial was planted in a stratified design, to take account of any gradients in the field with three replicates of each treatment. Two planting densities 10,000 and 15,000 pL ha^{-1} were evaluated with two mulch treatments (mulch film and control). Trial 1b was planted at Hackthorn following the same field trail design as trial 1a. However, due to field constraints, the mulch film treatment was replicated but the no film (control) only had one plot per treatment combination. Phenotypic measurements were collected on five plants in each plot in years 1, 2 and 3. Quadrat harvests were taken in years 2 and 3. Quadrats contained

TABLE 1 Description of the five mulch films tested in the trials.

Mulch film	Mulch film type	Thickness (μm)	Width (m)	Number of rows covered
Samco 'grey'	Oxo-degradable	7	1.3	2
Novamont 30	Biodegradable	30	1.3	2
Novamont 18	Biodegradable	18	1.3	2
Novamont 10	Biodegradable	10	1.3	2
Novamont 10 narrow	Biodegradable	10	0.45	1

a maximum of 10 plants per plot; therefore, the quadrat area was 10 and 6.6 m^2 for plots planted at 10,000 and 15,000 pL ha^{-1}, respectively.

3.3 | Trial 2 assessing the establishment and over winter survival of a pre-commercial seed-based hybrid established under 2 novel biodegradable mulch films and Samco grey

In 2019, a trial was planted at Hackthorn Lincolnshire (Terravesta Ltd.), to compare the effect of oxo-degradable mulch film, and novel BIS mulch films produced by Novamont, on the establishment and overwinter survival of *Miscanthus* plug plants. A randomized complete plot design trial was planted to assess three mulch films, 'Samco grey' 'pin hole' film, a 7 μm photodegradable mulch film and two BIS mulch films produced from bio-based resins Novamont 18 (18 μm thick) and Novamont 30 (30 μm thick). GRC 14 a pre-commercial intra-species seed-based *Miscanthus* hybrid was selected for the trial due to its promising characteristics as a seed-based hybrid of high yield and high seed set. The plug plants were produced by Bell Brothers nursery under glass and hardened off prior to planting. A 144-module tray, with a cell depth of 5 cm and cell volume of 45 cm^3 was used to produce the plug plants. Each plot was instrumented with two thermocouples linked to a Campbell CR1000 data logger (Campbell Scientific Ltd.) to record soil temperature at two depths, soil surface and −5 cm. Soil temperature was recorded from the day of planting and terminated for all plots once the mulch film surface had broken on one plot. Phenotypic measurements of stem height and stem count were taken in year 1, 2 and 3 plants were measured per plot, measurements were taken from the same position in each plot.

3.4 | Trial 3: Time and motion studies of commercial plug planting and mulch film laying

In 2020, commercial-scale un-replicated trial was established at Hackthorn Lincolnshire, (Terravesta Ltd.). Two fields totalling 9.4 ha were planted in mid-May with a four-row pelican plug planter and covered with Novamont 10 (10 μm) BIS mulch film. GRC 14 plantlets were produced by Bell Brothers nursery following the protocol used in 2019. Due to very poor establishment in 2020, the trials were removed with glyphosate and re-established in 2021 with an updated methodology.

Both fields totalling 9.4 ha were replanted in 2021. The plants were covered with Novamont 10 μm narrow mulch film (45 cm) produced by Samco, using a two-row Samco band mulch film layer. Each row of mulch film covers one row of plants rather two rows from conventional width mulch film. Stomp aqua was applied at 1.5 lt ha^{-1} when the mulch film was laid. A small block in one approx. 0.3 ha field was not covered with mulch film to provide a comparison of establishment and growth with and without mulch film although this was not replicated and therefore cannot be analysed statically.

3.5 | Statistical analysis

Statistical analysis was performed in R (R Development Core Team, 2015) with statistics from car package (Fox, 2019). Trial 1a and 1b establishment, overwinter survival, yield, height and stem count results used multifactor unbalanced ANOVAs to find differences between film treatments, hybrid, density and year. First year data were analysed separately with the same methodology as this data more replicate plots and contained more hybrids than later years. While trial 2 (the Novamont vs. Samco) was analysed with one-way ANOVAs to assess whether film type had a significant effect on establishment, survival, height and stem count. Whenever data were not normally distributed, it was simply transformed using a method stated in the text to provide a Gaussian distribution, percentage establishment was always arcsine transformed. Where significant, interactions are reported otherwise, they were excluded to simplify the model. Tukey's HSD from the agricolae package (de Mendiburu, 2021) was used for all post hoc tests.

4 | RESULTS

4.1 | Trial 1a

4.1.1 | Establishment success and overwinter survival

Average establishment success rates were higher in seed plugs 89.9%, than in rhizome planted GRC 9 74.9%. Mean transplant establishment success rates were 91.5% and 81.9% for plots covered with Samco Grey mulch film and control plots, respectively. Mulch film increased average establishment by 9.5%, 7.4%, 8.2%, 2.3% and 20.5% for GNT 1, GNT 2, GNT 4, GRC 9 and GRC 10, respectively. Both mulch film and hybrid significantly affected establishment $p<0.001$ and $p<0.05$, respectively. Average establishment was 86% and 87% for planting density of 10,000 and 15,000 pL ha^{-1}, respectively. Planting density did not significantly affect establishment ($p = 0.72$).

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Overwinter survival was higher for all hybrids when covered with mulch film. Average over winter survival was 68% and 48.3% for plots covered with mulch film at planting and control plots, respectively. Mulch film significantly affected overwinter survival $p<0.001$. There were overwinter losses for all hybrids in both mulch film covered plots and control plots (Figure 1). Hybrid significantly affected overwinter survival $p<0.001$. Over winter survival varied between hybrids ranging from 46.4% to 82.6% for plots covered with mulch film compared with 20.2% to 68% without mulch film at planting (Figure 1). Mulch film had larger impact on overwinter survival of the seed-based hybrids than rhizome-established *Mxg*. Average overwinter survival was 10.5%–43.6% higher for seed plugs covered with mulch film at planting than control plots compared with 3.3% for *Mxg* established from rhizome. There was a significant hybrid mulch film interaction on establishment $p<0.01$. Planting density did not significantly affect overwinter survival $p = 0.97$. Overwinter survival was 58.8% and 58.8% for planting density of 10,000 and 15,000 pL ha^{-1}.

4.1.2 | Phenotypic measurements

Stem count and stem height were measured at end of the growing season (late October) in years 1, 2 and 3 (Figure 2). Due to poor over winter survival in year of GNT 1 and GRC 10 both hybrids were discounted from measurement in years 2 and 3.

Stem height

Average stem height at the end of the growing season was higher for plots covered with film for all 3 years however the increase diminished each year. In year 1, average stem height was 56.1 and 39.8 cm for plots covered with mulch film and control plots. Whilst in years 2 and 3 stem height was 105 cm and 189 cm for plots covered with mulch film and 93.6 and 183.9 cm and control plots. Mulch film significantly affected shoot height in Year 1 $p<0.001$ but did not in years 2 and 3 $p = 0.53$. Planting density did not have a significant effect on stem height in year 1 or years 2 and 3 $p = 0.07$ and $p = 0.59$, respectively. Stem height in plots planted at a density of 10,000 pL ha^{-1} was 45.6, 93.8 and 183.9 cm for years 1, 2 and 3, respectively, and 100.3, 104.75 and 189 cm in years 1, 2 and 3 planted in a density of 15,000 pL ha^{-1}. Hybrid significantly affected stem height in year 1 $p<0.001$ and in years 2 and 3 $p<0.001$. Average stem height was 56.5, 37.8, 52.2, 46.2 and 47.9 cm for GNT 1, GNT 2, GNT 3, GNT 4 and GRC 9, respectively, in year 1. In year 2 stem height was 68.4, 78.5 and 151 cm while in year 3 it was 143.5, 158 and 257.8 cm for GNT 2, GNT 4 and GRC 9, respectively.

Stem count

Mulch film significantly increased stem count per plant in year 1 $p<0.001$ and years 2 and 3 $p<0.05$. Stem count was higher for all hybrids when covered with mulch film compared with control plots (Figure 2). Mean stem count per plant in year 1 was 14.7 and 11.4, 26.4 and 21.6 in year 2 and 43.6 and 37.5 in year 3 for plots covered with mulch film and control plots, respectively.

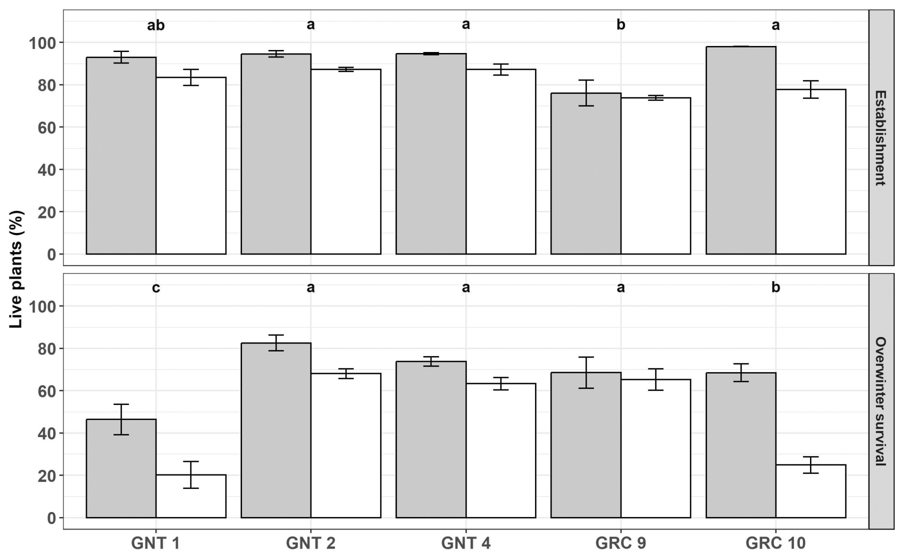

FIGURE 1 First year establishment of four seed-based *Miscanthus* hybrids GNT 1, GNT 2, GNT 4 and GRC 10 using plug plants and a clonal hybrid by rhizome (GRC 9) broken down into two stages: (a) establishment and (b) overwintering survival with mulch film (grey) and without (white). Trial 1A at Aberystwyth. Error bars show SE $n = 3$. Letters show Tukey's post hoc test.

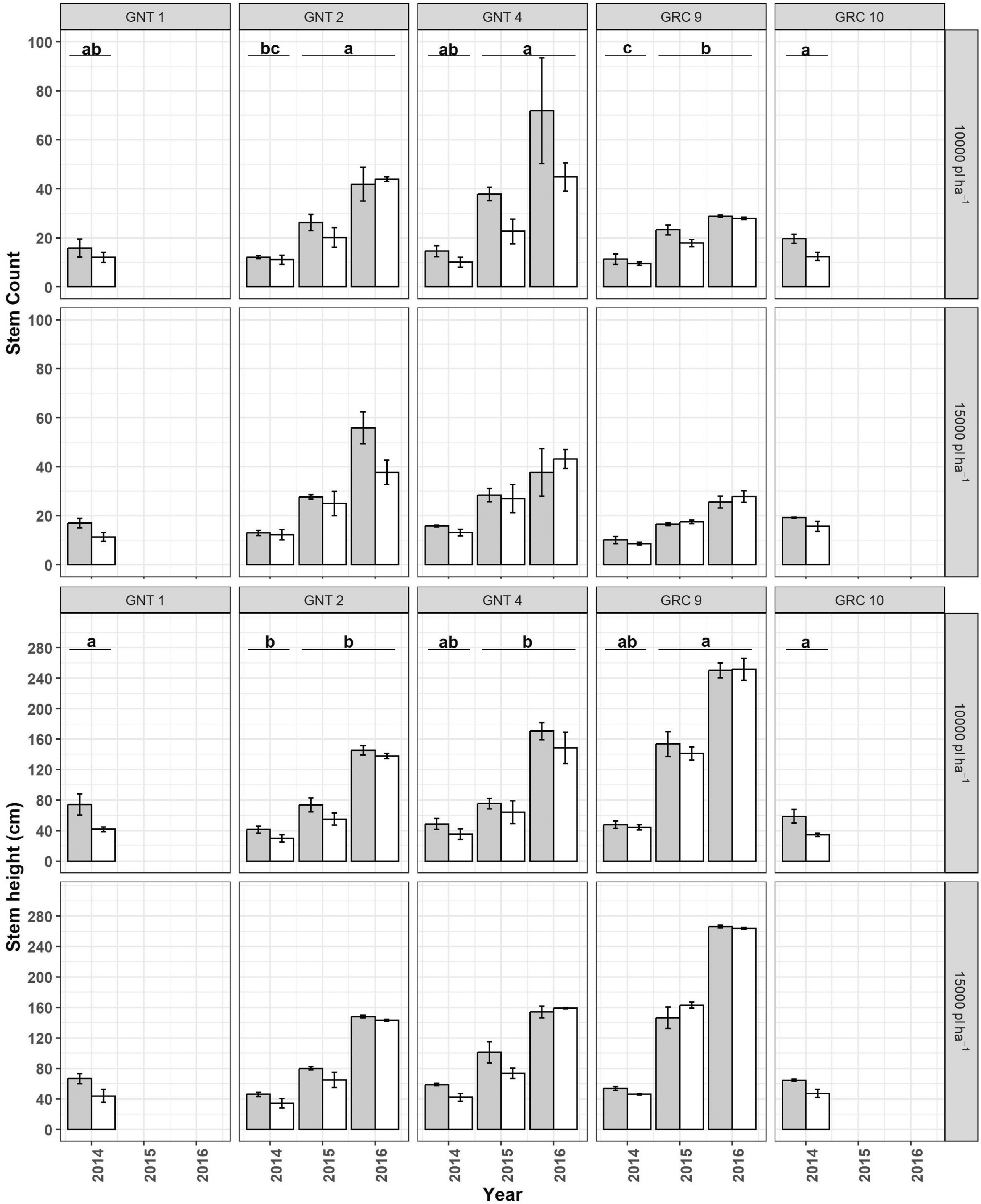

FIGURE 2 Mean stem height (cm) and mean stem count of four seed-based *Miscanthus* hybrids GNT 1, GNT 2, GNT 4 and GRC 10 established from plug plants and GRC 9 established by rhizome covered with mulch film at planting (grey) and control (white) planted at three densities 10,000 and 15,000 pL ha^{-1} measured at the end of the growing season. Error bars show SE $n = 3$. Letters show Tukey's post hoc test for year 1 (2014) and years 2 and 3 combined (2015 and 2016).

Average stem count was 14.6, and 14.9 for mulch film covered plots and 10.9 and 12.1 for control plots in year 1 at planting densities of 10,000 and 15,000 pL ha^{-1}, respectively. Stem count in film covered plots increased to 24.8 and 23.5 year 2 and 43.7 and 37.3 in year 3 for planting densities of 10,000 and 15,000 pL ha^{-1}, respectively. Planting density did not significantly affect stem count in year 1 $p = 0.31$ or in years 2 and 3 $p = 0.18$. Stem count per plant varied between hybrid, in all years GRC 9 had the lowest stem count and years 2 and 3 GNT 4 had the highest stem count. Average stem count per plant was 14, 12.1, 13.3, 9.8 and 16.7 GNT 1, GNT 2, GNT 4 and GRC 9 and GRC 10, respectively, in year 1. In year 2, stem count was 24.7, 29.8 and 18.3 and in year 3 it increased to 44.9, 48 and 26.7 for GNT 2 and GNT 4 and GRC 9, respectively. Hybrid significantly affected stem count in year 1 and years 2 and 3, $p < 0.001$ and $p < 0.001$.

Yield

Dry matter yield (kg dm ha^{-1}) was measured in years 2 and 3 (2016 and 2017) (Figure 3). Mulch film significantly affected yield $p < 0.01$. Yield in plots covered with mulch film was higher in both 2016 and 2017 than in control plots. Average dry matter yield was 3958 and 12,419 kg ha^{-1} for mulch film covered plot in 2016 and 2017, respectively. In comparison, yield of control plots was 2939 and 10,698 kg ha^{-1} in 2016 and 2017, respectively. Mulch film had a greater effect on the yield of GNT 4 and Mxg than it did on GNT 2 (Figure 3). However, hybrid did not have a significant effect on yield $p = 0.58$.

Planting density had a significant effect on yield $p < 0.01$, higher planting density (15,000 pL ha^{-1}) had higher yields in both film covered and control plots in both years. Yield was 984 and 1699 kg ha^{-1} higher in plots covered with mulch film planted at 15,000 pL ha^{-1} than those planted 10,000 pL ha^{-1} in 2016 and 2017, respectively. The increase in yield with increasing density was higher in control plots than mulch film covered plots. Dry yield was 1699 kg ha^{-1} and 3151 kg ha^{-1} higher in control plots planted at 15,000 pL ha^{-1} compared with those planted 10,000 pL ha^{-1} in 2016 and 2017, respectively.

4.2 | Trial 1b

4.2.1 | Establishment

Mean establishment ranged from 93.1% to 99.1% in film covered plots and 75% to 96.2% in control plots

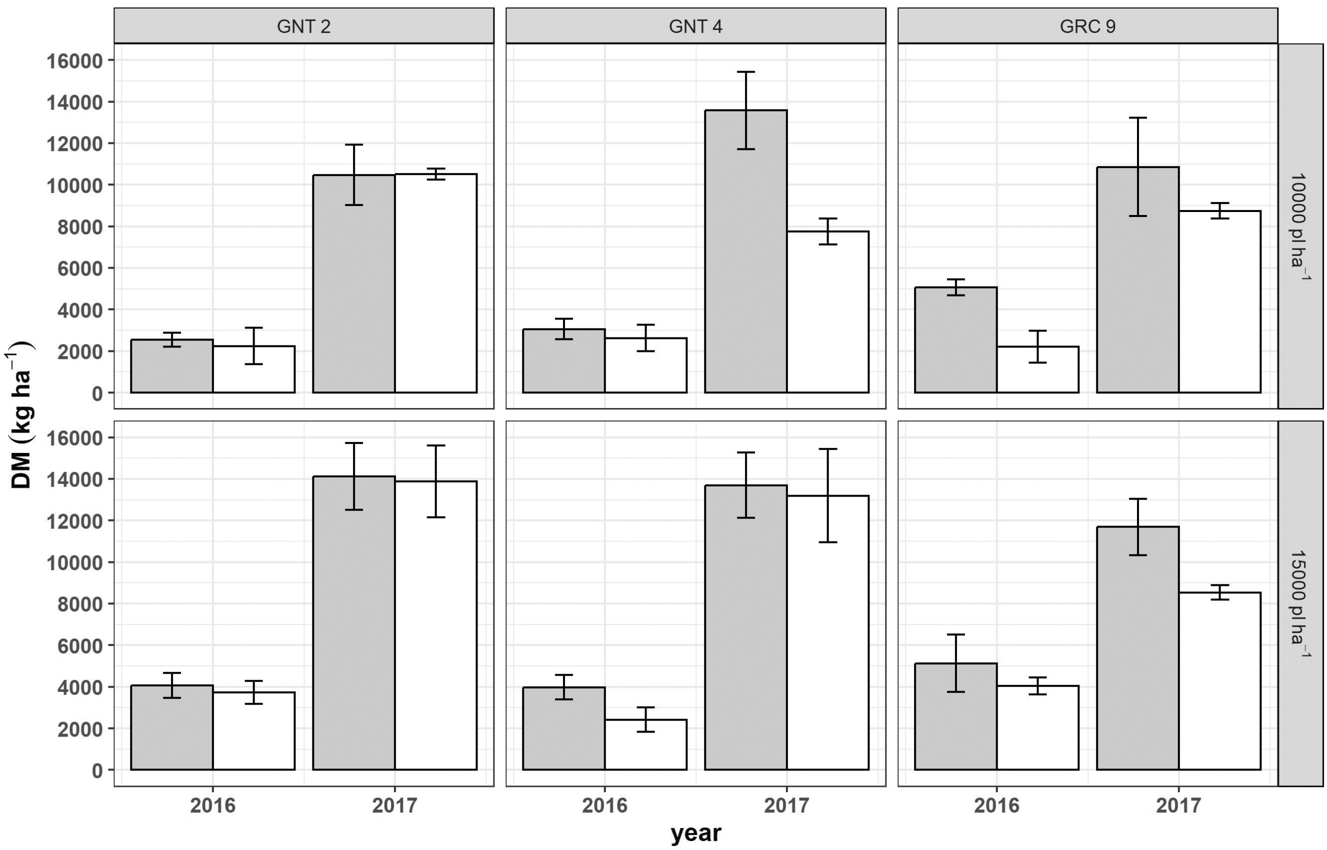

FIGURE 3 Mean dry matter yield (kg) of GNT 2, GNT 4 and GRC 9 at Aberystwyth covered with mulch film (grey) and control, without mulch film (white) in year 2 (2016) and year 3 (2017) planted at 2 densities 10,000 and 15,000 pL ha^{-1}. Error bars show SE $n = 3$.

(Figure 4) and was higher for all hybrids when established under mulch film compared with control treatment. Mulch film significantly affected establishment $p < 0.01$. Establishment from seed-based plug plants (GNT 1, GNT 2, GNT 4 and GRC 10) was higher than plots established from rhizome (GRC 9) 97.3% and 93.1%, respectively. However, in plots established without mulch film establishment was higher for plots planted with rhizome than plots planted with seed-based plug plants 90.9% and 82.2%, respectively. Hybrid and planting density did not significantly affect establishment $p = 0.27$ and $p = 0.52$, respectively. The impact of mulch film on establishment varied with hybrid. Mulch film increased mean establishment by 1.3%, 2.2%, 15.9%, 19.3% and 24.1% for GNT 4, GRC9, GRC 10, GNT 1 and GNT 2, respectively. There was a significant interaction between hybrid and mulch film $p < 0.01$.

4.2.2 | Phenotypic measurements

Stem height

Mean stem height was 92.6, 123.8 and 177 cm in plots covered with mulch film in years 1, 2 and 3, respectively. Stem height was lower for all 3 years in control plots 65.5, 105.1 and 176.8 cm for years 1, 2 and 3. Increase in stem height in film covered plots decreased with year from 27.1 cm in year 1 to 0.2 cm in year 3. Mulch film significantly increased stem height in year 1 $p < 0.001$ but did not in years 2 and 3 $p = 0.14$. Hybrid significantly affected stem height in year 1 $p < 0.01$ and in years 2 and 3 $p < 0.001$. Average stem height ranged from 66.2 to 87.5 cm, 74.6 cm to 145.7 cm and 110.7 to 250.7 cm in years 1, 2 and 3, respectively (Figure 5). GNT 2 had the shortest stems in all years; however, whilst GRC 10 had the tallest stems in year 1, GRC 9 had the tallest stems in years 2 and 3. Mean stem height was 78.5 and 79.6 cm in year 1, 119.3 and 109.7 cm in year 2 and 179.3 and 174.6 cm in year three for planting densities of 10,000 and 15,000 pL ha^{-1}, respectively. Planting density did not have a significant effect on stem height year 1 or years 2 and 3 $p = 0.17$ and $p = 0.13$, respectively.

Stem count

Mean stem counts per plant ranged from 5.4 to 8.5, 28.5 to 42 and 37.4 to 75.3 in film covered plots for years 1, 2 and 3, respectively. Generally, control plots had lower stem counts than film covered plots ranging from 3.9 to 5.2, 16 to 33.8 and 26.8 to 64.7 for years 1, 2 and 3, respectively. Mulch film significantly affected stem count in year 1 $p < 0.001$ and in years 2 and 3 $p < 0.01$. Planting density did not significantly affect stem count in year 1 $p = 0.91$ but did in years 2 and 3 $p < 0.001$. In all 3 years, mean stem count was higher in plot planted at 10,000 pL ha^{-1} than plots planted at 15,000 pL ha^{-1}. Stem count was 6.2, 35.3 and 58.5 for years 1, 2 and 3, respectively in plots planted at 10,000 pL ha^{-1} compared with 5.8, 28.3, and 40.8 for years 1, 2 and 3, respectively, in plots planted at 15,000 pL ha^{-1}. Hybrid significantly affects stem count in both year 1 and years 2 and 3 $p < 0.001$ and $p < 0.001$. In all 3 years, GRC 9 had the lowest stem count per plant in all year, 4.6, 22.3 and 32.1 in years 1, 2 and 3, respectively. In year 1, GRC 10 had the most stems (6.8) but in years 2 and 3 GNT 2 had the highest stem count, 37.9 for year 2 and 70 for year 3.

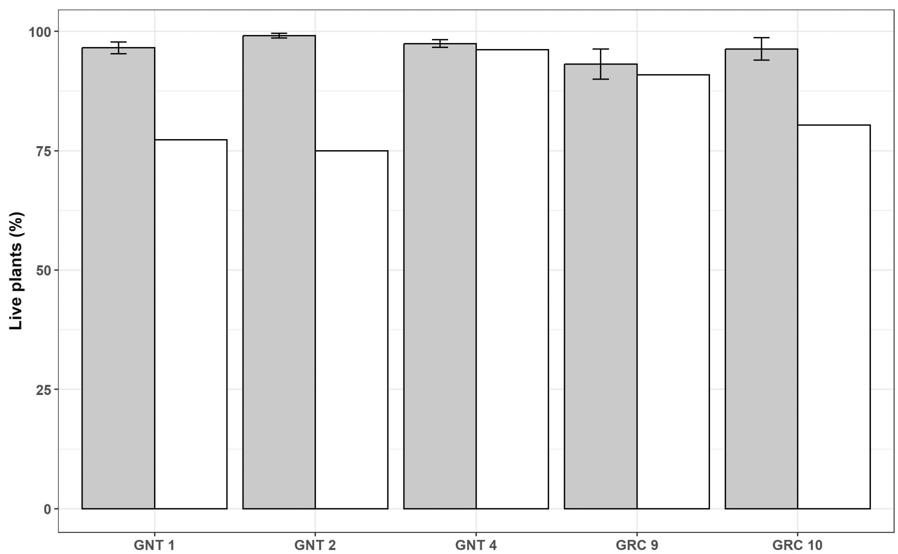

FIGURE 4 Mean establishment at Hackthorn of four seed-based *Miscanthus* hybrids GNT 1, GNT 2, GNT 4 and GRC 10 established from plug plants and GRC 9 established by rhizome covered with mulch film at planting (Grey) and without mulch film (white). Error bars show SE $n = 3$.

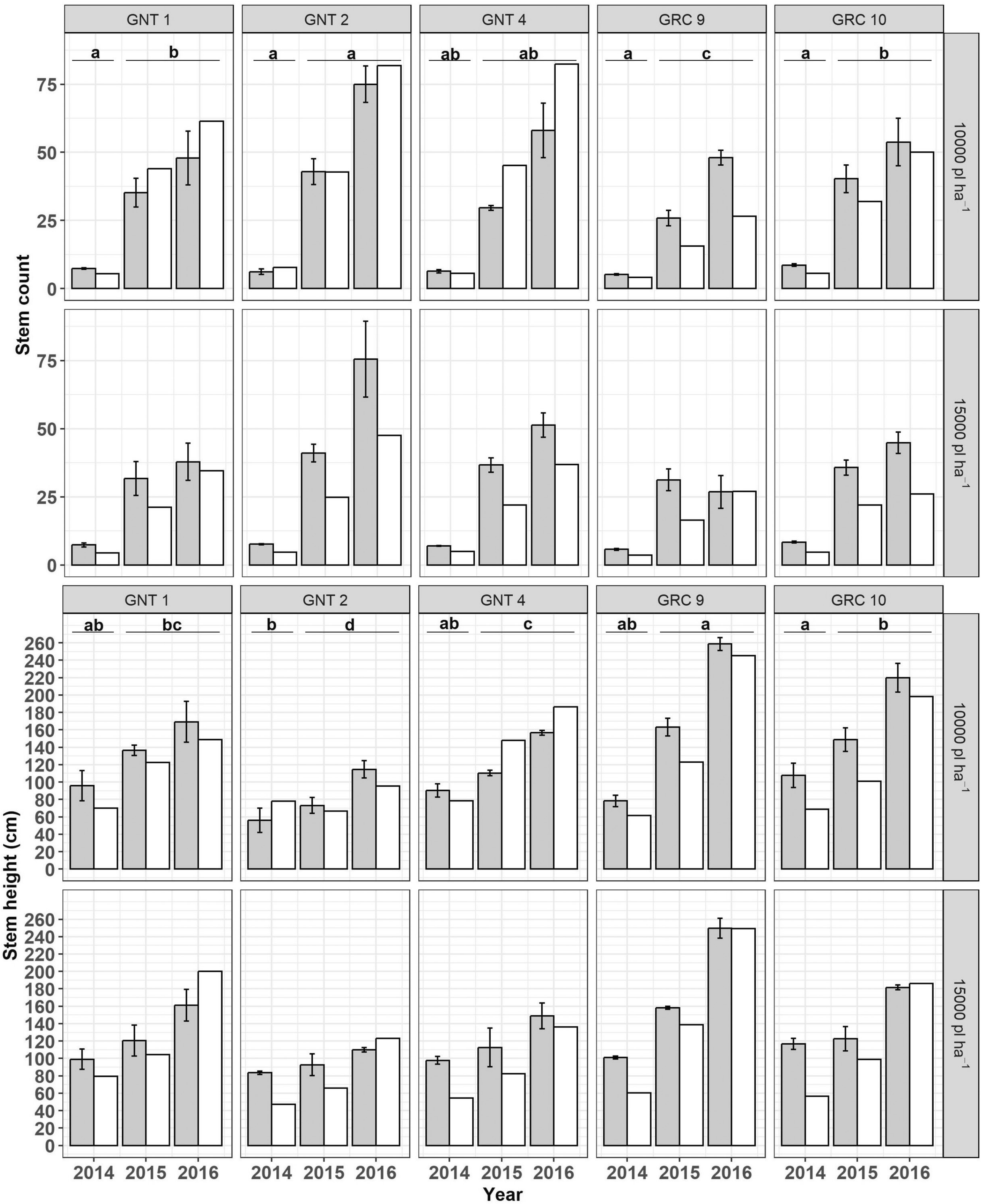

FIGURE 5 Mean Stem height (cm) and mean stem count of four seed-based *Miscanthus* hybrids GNT 1, GNT 2, GNT 4 and GRC 10 established from plug plants and GRC 9 by rhizome covered with mulch film at planting (grey) and without mulch film (white) planted at two densities 10,000 and 15,000 pL ha^{-1} measured at the end of the growing season. Error bars show SE $n = 3$. Letters show Tukey's post hoc test for year 1 (2014) and years 2 and 3 combined (2015 and 2016).

Yield

Dry matter yield (kg ha^{-1}) was measured in years 2 and 3 (2016 and 2017). Yield ranged from 3502 to 8478 kg ha^{-1} in 2016 and from 6378 to 15,959 kg ha^{-1} in 2017 (Figure 6). Mulch film significantly affected yield $p < 0.001$. Yield in plots covered with mulch film was higher in both 2016 and 2017. In 2016, mean dry matter yield was 6954 and 4439 kg ha^{-1} for mulch film covered and control plots, respectively. In 2017, yields increased to 12,257 and 9643 kg dm ha^{-1} in mulch film covered and control plots, respectively.

Planting density had a significant effect on yield $p < 0.01$, higher planting density (15,000 pL ha^{-1}) had higher yields for film covered plots in both years. Dry matter yield was 828 and 1387 kg ha^{-1} higher in plots covered with mulch film planted at 15,000 pL ha^{-1} than those planted 10,000 pL ha^{-1} in 2016 and 2017, respectively. In 2016, yield in control plots yield did not increase with density, yield was 4490 and 4388 kg ha^{-1} for planting densities of 10,000 and 15,000 pL ha^{-1}, respectively. However, in 2017, yield in control plots did increase with density from 9441 kg ha^{-1} for 10,000 pL ha^{-1} to 9845 kg ha^{-1} for 15,000 pL ha^{-1}.

Hybrid significantly affected yield $p < 0.05$. In 2016, GNT 2 had the lowest mean dry matter yield for film covered 5569 kg ha^{-1} and control plots 4094 kg ha^{-1}. Whilst in 2017 GNT 4 had lowest mean yield of 8680.9 kg ha^{-1} and 7216 kg ha^{-1} for film covered and control plots, respectively. The highest-yielding hybrid varied between years and mulch film treatment. In 2016, GRC 10 had the highest yield, 8935 kg ha^{-1} for film covered plots and GNT 4 had the highest yield for control plot, 4831 kg ha^{-1}. In 2017, GRC 9 produced highest yield is mulch film covered plots, 15,431 kg ha^{-1} whilst GNT 1 yielded highest in their control plots, 11,004 kg ha^{-1}. There was a significant interaction between hybrid and year $p < 0.01$.

4.3 | Trial 2

4.3.1 | Soil temperature and thermal time

Soil temperature was measured at two depths soil surface and −5 cm (Figure 7) in each plot. Max temp at soil surface was 46.1, 46.4 and 53.2°C for Samco Grey, Novamont 18 and Novamont 30 mulch films, respectively (Figure 7). Min temp at soil surface was 2.4, 3 and 1.2°C for Samco Grey, Novamont 18 and Novamont 30 mulch

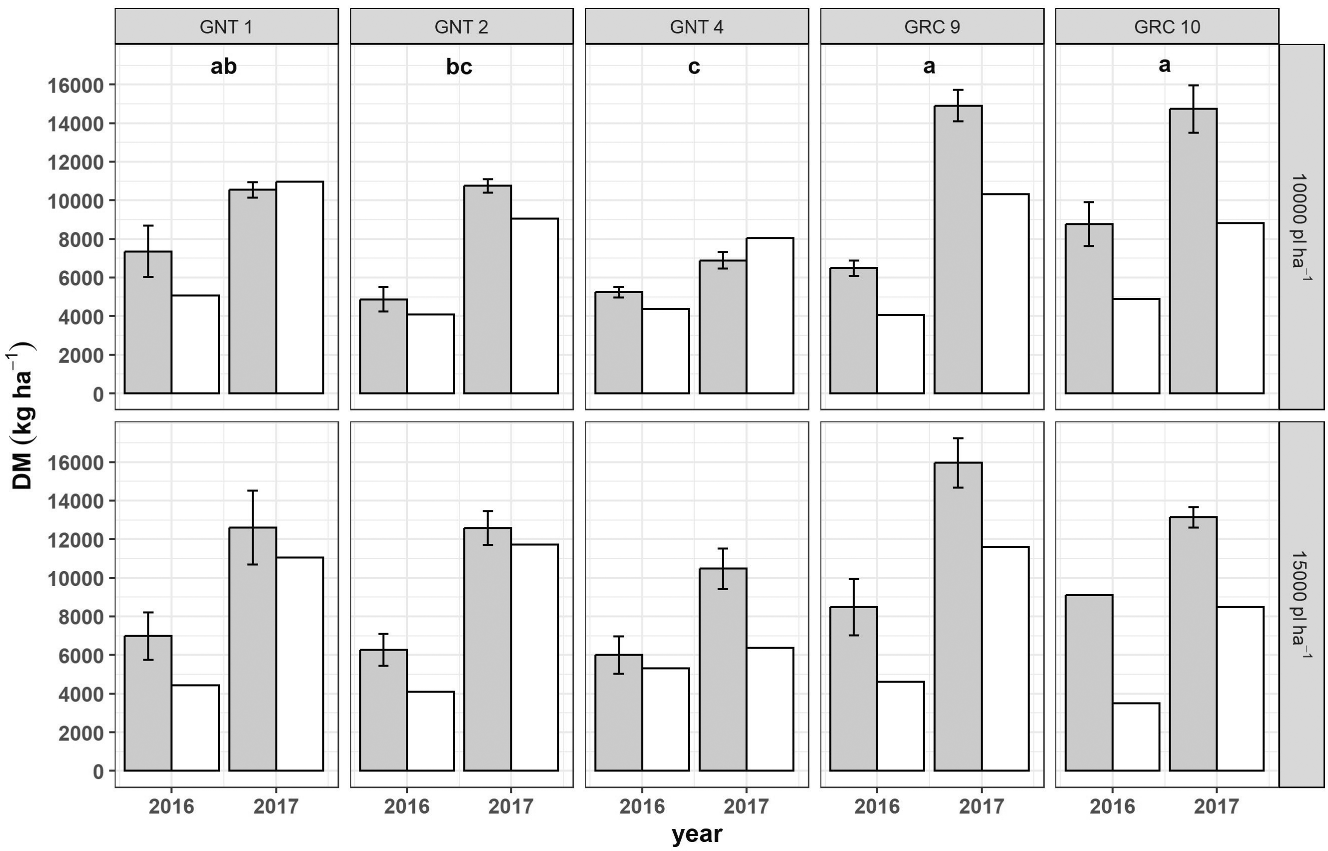

FIGURE 6 Mean dry matter yield (kg) of GNT 1, GNT 2, GNT 4 and GRC 10 established from plug plants and GRC 9 from rhizome at Hackthorn covered with mulch film (grey) and control, without mulch film (white) in year 2 (2016 and year 3 (2017) planted at 10,000 and 15,000 pL ha^{-1}. Error bars show SE $n = 3$. Letters show Tukey's post hoc test.

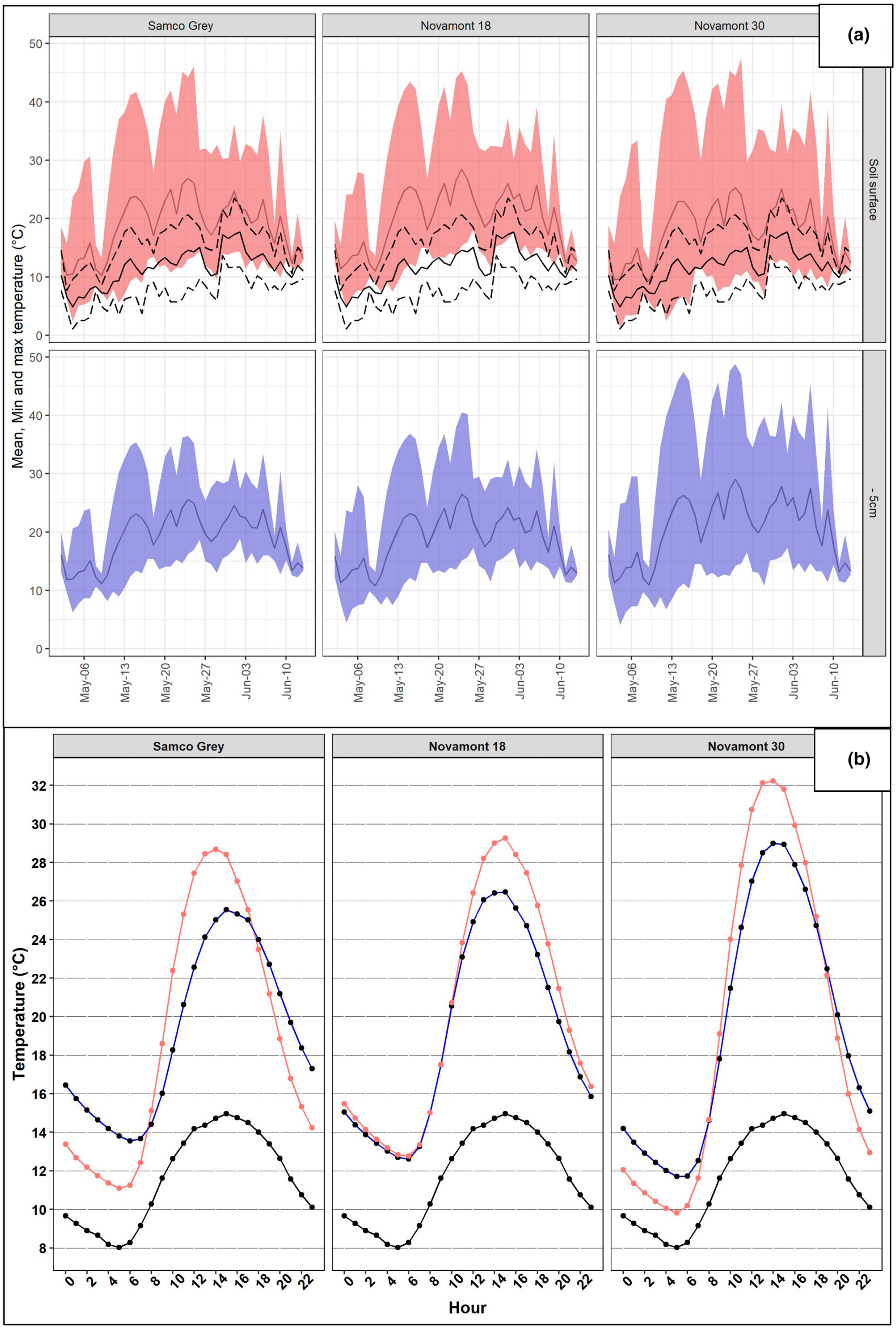

FIGURE 7 (a) Min, Max and Mean temperature under one photodegraded mulch film (Samco Grey) and two novel BIS mulch films (Novamont 18 and Novamont 30) at two depths soil surface (red) and −5 cm (blue) from 2 May 2019 to 15 June 2019. Black lines show Min, Max and Mean air temperature at RAF Scampton 3 miles from field trial location. (b) Mean hourly soil temperature (°C) under one photodegraded mulch film (Samco Grey) and two novel BIS mulch films (Novamont 18 and Novamont 30) at two depths soil surface red, −5 cm blue and black air temperature at 2 m for the period 2 May 2019 to 15 June 2019. Air temp data from RAF Scampton.

films, respectively. Soil temperatures at −5 cm varied less than those at the soil surface. Minimum and maximum soil temps at −5 cm were 6.2 and 36.5°C, 5.6 and 38.7°C, and 4.1 and 42.7°C for Samco Grey, Novamont 18 and Novamont 30, respectively.

Thermal time was calculated as accumulated degree days above the base temperature of 8.5°C. Accumulated degree days above a base of 8.5°C was highest under Novamont 30 at 583 while accumulated degree days under Samco grey and Novamont 18 were 509 and 500, respectively. Comparatively accumulative degree days above a base of 8.5°C without mulch film was 161. Therefore, there was an increase of 339, 349 and 422 degree days through covering plants with Novamont 18, Samco Grey and Novamont 30 mulch films, respectively.

4.4 | Establishment and overwinter survival

Mean establishment was higher in plots covered with Samco grey mulch film 83.7% compared with plots covered with Novamont 18 and Novamont 30, 67.4% and 72%, respectively. However, mulch film type did not have a significant effect on establishment $p = 0.21$. Overwinter survival was also higher in plots covered Samco grey 75.5% compared with Novamont 18 and Novamont 30, 61.7 and 66.3%. Over winter losses were lower for both Novamont 18 and Novamont 30 mulch film 5.7 and 5.7%, respectively, than Samco Grey 8.2%. Mulch film type did not significantly affect overwinter survival $p = 0.97$.

4.5 | Stem count and height

In year 1, average stem count per plant was 11.4, 11 and 12 for Novamont 18, Novamont 30 and Samco grey mulch films, respectively. There was no significant effect from mulch film type on stem count $p = 0.96$. Average stem height per plant was 43.25, 39.25 and 40.66 cm Novamont 18, Novamont 30 and Samco grey mulch films, respectively. There was no significant effect from mulch film or block on stem height $p = 0.84$.

4.6 | Trial 3: Commercial tests of biodegradable mulch film

In 2020, commercial scale trials totalling 9.4 ha were planted at Hackthorn, Lincoln to test the efficacy of combining techniques widely used in vegetable production and *Maize* production at commercial scale to establish seed-based *Miscanthus* plugs and test BIS mulch film at commercial scale. The 144 module trays performed poorly in the pelican planter due to the flexibility of the single use tray and the planter carousel could not be utilized which significantly slowed planting. The approximate area planted each day was around 1.5–2 ha. Immediately after planting mulch film was laid to limit soil moisture losses and protect the seedlings from low temps overnight. The area of mulch film laid each day was 1.5–2 ha and therefore mulch film laying pace matched planting pace. Soil texture varied in some areas of the field due to the size of the trial. In areas with heavier soil mulch film laying speed had to be reduced to ensure the edges of the film were adequately covered. The trials were planted in mid-May once the winter sown plug plants were mature enough for planting. Soil moisture at planting was low due limited rainfall in the weeks preceding planting. Rainfall was also limited in the weeks following planting and establishment was very low. Due to the very low establishment, establishment counts were not taken, the trial was removed with glyphosate once the mulch film had degraded.

Following the establishment failure in 2020, the plug production and establishment methodologies were reviewed and updated prior to replanting the trials in 2021. Two potential challenges to establishment were identified (1) planting date and resultant soil moisture/limited rainfall following planting and (2) mulch film reducing the impact of rainfall after planting due to a large surface area of the field covered with mulch film.

To address these challenges, plug plants were sown in the summer and overwintered prior to planting. Rigid, reusable 216 modules trays were used. A two-bed narrow film layer from Samco using 45 cm wide mulch film that covered 1 row of plants was tested. Summer sowing produced mature plants earlier than winter sowing and the trial was planted approx. 3 weeks earlier in 2021 than in 2020. As such soil moisture was higher at planting in 2021 than 2020 and rainfall following planting was higher. Rigid module trays allowed the planter carrousel to be used which increased the planting speed to 3 ha per day (Figure 8b). The narrow mulch film layer tested in 2021 was able to lay mulch film faster than the film layer used in 2020. Therefore, although it only covered 2 rows rather than 6 it could keep pace with the planter and cover approx. 3 ha per day (Figure 8c,d). Establishment was much higher in 2021 than 2020 and complete canopy cover was present for most of the field by the end of the first growing season. Establishment counts taken from two location within the field showed establishment was greater >90% as was subsequent emergence after the first winter.

FIGURE 8 (a) Summer sown 216 module plug b) 1.3 m wide 10 µm Biodegradable in soil (BIS) mulch film, Hackthorn 2020, (c) Pelican planter utilizing rigid 216 module trays and Samco narrow mulch film layer, and (d) 45 cm narrow single row mulch film, Hackthorn 2021.

5 | DISCUSSION

Significant upscaling of biomass crops is required to meet the goals for carbon reduction (CCC, 2020) and to provide bio-based economies with sufficient sustainable feedstock for green manufacturing. However, great care must be taken to avoid displacing food production while upscaling biomass production. Therefore, techniques to establish biomass crops on marginal lands that ensure high yield and are economically viable must be developed. Un-favourable environmental conditions during the establishment phase can have negative effects on crop yields for both annual and perennial crops. Early plant development and stand establishment of perennial crops can impact harvestable biomass yields for multiple years (Dauber et al., 2015; Zimmermann et al., 2014). Seed-based *Miscanthus* hybrids offer much higher multiplication factors than clonal propagule production (Clifton-Brown et al., 2017). To date, direct sowing has had very limited success (Ashman et al., 2018; Christian et al., 2005), crop establishment with indirect sowing via glasshouse grown seedlings in plugs has been prioritized.

5.1 | Mulch film impacts on transplant survival

Clear plastic mulch films applied after sowing or planting have been shown to de-risk seedling establishment of a wide range of crops and improve yield around the globe. These are particularly effective in cooler climates on thermophilic crops such as maize because they both increase the temperature and reduce the risk of desiccation (Easson & Fearnehough, 2000; Farrell & Gilliland, 2011 #33). In Ireland, O'Loughlin et al. (2017) found that mulch film increased survival and accelerated early plant development of $M \times g$ significantly. In experiments reported in this paper mulch film effects on *Mxg* (GRC 9) rhizomes slightly increased survival after transplanting in at ABR and at HCK (2.3 and 2.2%, respectively). The overall benefit from the film coverings were greater for the establishment of seeded hybrids (13%–16.6%) via plugs plants than rhizome propagated *Mxg*.

The economic impact of mulch film on establishment can be determined by the additional propagules required to reach the required field density (O'Loughlin et al., 2017). Due to the small differences in establishment of GRC 9 between the mulch film treatments at both sites the reduction in propagules required due to mulch film was negligible; however, this was not true of the seed-based hybrids. The morphology of the seed-based hybrids bred at IBERS differs from GRC 9 and a field density of at least 15,000 pL ha^{-1} is expected to be necessary to reach peak yields. An additional 1920 to 3330 and 570 to 3750 propagules would need to be planted depending on hybrid at ABR and HCK, respectively, to achieve field density of 15,000 pL ha^{-1} if planted without mulch film compared with 250–1050 and 135–1035 at ABR and HCK, respectively, with mulch film.

Establishment and the cost of additional plantlets per hectare is only part of the justification for using mulch films. Poorer establishment will lead to patchy crops, reduction in yields and increases in costs of additional inputs such as herbicides. Establishment is an early sign of success; however, overwintering success can also significantly affect the number of live plants per hectare in mature stands in year 2 and onwards and therefore yield (Dauber et al., 2015; Zimmermann et al., 2014).

Two out the four seed-based hybrids GNT 1 and GRC 10 planted at ABR had poor to overwintering success. GNT 1 had very low overwinter success in the first winter, irrespective of whether it had been covered with mulch film or not. Therefore, it can be concluded that GNT 1 is not tolerant of the growing environment at this site in Mid Wales. GRC 10 also had very poor overwinter survival in control plots (24.9%), but overwinter survival was much higher (68.5%) for plots covered with mulch film. This confirms mulch film will de-risk the planting of some hybrids at sites with less favourable growing conditions. It is likely that the increased temperature and moisture under the mulch film at ABR promoted greater rhizome development in plots of GRC 10 covered with mulch film, giving it greater tolerance to frosts.

5.2 | Impacts of mulch film on yield traits and final yield

In the first year after planting, as expected from other studies (Olave et al., 2017, O'Loughlin et al., 2017 #32), mulch films stimulated higher stem growth rates leading to taller plants at the end of the growing season. However, these strong effects reduced in year 2, and by year 3 the difference in height was not significant between those that were covered with mulch film and those that were not. In contrast to these diminishing impacts of mulch film overtime, height differences between hybrids remained statistically significant in all 3 years. Olave et al. (2017) also found that increased tillering was the main effect of mulch film on *Mxg*. This suggests that the extra warmth under the mulch film increased rhizome development causing a compound interest effect on growth which is carried through into successive years. As with stem height, hybrid had a significant effect on stem count at ABR and HCK in all 3 years, showing the importance of hybrid selection. Standard *Mxg* (GRC 9 in this study) had lowest stem counts per plant but the tallest stems in year 3 at both sites. This demonstrates the strength of the genetic control of phenotypic traits: 'hybrid' is a larger determinant of the mature phenotype than the environmental conditions caused by different sites and agronomic methods.

At both sites, mulch film application significantly increased harvestable biomass yield and this enhancement affect persisted for all 3 years of the trial. This has also been observed in Ireland in previous trials (O'Loughlin et al., 2017). In our trials, the impact of mulch film on yield varied between different hybrids: with a limited effect on GNT 2 and GNT 4 and a greater effect on GRC 9 at ABR and GRC 9 and GRC 10 at HCK.

Assuming a field gate price of £75 Mg^{-1} (Hastings et al., 2017) and assuming a moisture content of 15% additional income per hectare in the third-year harvest would be £385 and £456 for GRC 9 and GRC 10, respectively, at HCK and £226 for GRC 9 at ABR. Increasing planting density produced significant increases to yield in the first 3 years yet often did not significantly affect stem heights or stem counts. It is well known that different perennial crops have different optimum planting densities. Planting densities above optimum can have negative effects on long-term yield, increased disease and or produce thinner stems that are more likely to lodge (Zhang et al., 2014). Due to lack of negative effect at the higher planting density in the trials reported here we suggest that the optimum planting density could be higher than 15,000 pL ha^{-1} but could only be verified with longer-term yield observations greater than 6–10 years.

Mulch film increased establishment and the growth parameters measured at both sites; however, the performance varied between hybrids and between sites. It is well known there is a strong genotype × environment interaction affecting both establishment and yield for many plants. Our results concur with those found by (Awty-Carroll et al., 2023), establishment and yield over the first 3 years of a range *Miscanthus* hybrids established by seed-based plug plants and rhizome that included GRC 9 and GRC 10 was significantly affected by location when tested at seven sites across Europe.

Yield was increased by mulch film in both years 2 and 3 suggesting that mulch film could reduce the establishment period for the *Miscanthus* hybrids tested. Mulch film clearly improved established percentage and growth; however, as only a 3-year trial it cannot be confirmed that mulch film shortened the establishment period. Where mulch film was most effective was in significantly de-risking, this establishment method in unfavourable conditions. Furthermore, through using mulch film it will be possible to establish *Miscanthus* crops earlier in spring due to the protection provided against low temperatures. Not only will this extend the growing season in Year 1 but allow the crops to establish prior to summer droughts.

5.3 | BIS mulch film trial

Mulch film has been called the 'white revolution' (Liu et al., 2014) and has extended the geographic range in which some crops can be grown either through improving soil temperature or reducing soil moisture loss (Easson & Fearnehough, 2000; Farrell; Kwabiah, 2005; O'Loughlin et al., 2017). However, it is essential that non degradable mulch films are completely recovered and disposed of properly, or better recycled, otherwise mulch film fragments (including microplastics) could accumulate in soil with negative impacts on the environment (Li et al., 2022; Liu et al., 2014; Yang et al., 2022). BIS mulch film made from bioplastics provide an alternative to non-degradable mulch film and to oxo-degradable mulch films.

Two BIS mulch films produced from Mater-BI bioplastic developed by Novamont Ltd. were tested alongside Samco grey, oxo-degradable mulch film. Soil surface temperature and soil temperature at a depth of 5 cm were higher for the BIS films compared to Samco grey. This was expected as both the BIS films were thicker than Samco grey. Soil surface temperature peaked at 53°C under the thickest film Novamont 30 and was only 7.1°C higher than the coolest film, Samco grey 46°C. While these peak temperatures are extreme, the duration is limited no damage to the shoot meristems were recorded because they are protected from the extreme temperatures just below the soil surface. Key traits of establishment, overwinter survival and phenotypic traits of stem height and count did not vary significantly between the mulch films and therefore the BIS, bioplastic-based mulch films were shown to offer a viable alternative to petroleum-based oxo-degradable films.

5.4 | Development of commercial plug planting protocols

Scalable protocols for *Miscanthus* plug planting are required for the commercial role out of seed-based *Miscanthus* hybrids to allow rapid upscaling. For plug planting to be a commercially viable alternative to establishment from rhizome, the protocols developed must (1) ensure high establishment and overwinter survival, (2) be achievable at large scale and at a minimum plant similar areas per day as rhizome and (3) be economically viable.

In 2020, time and motion studies were undertaken develop the plug to field protocols. Over nine hectares were established from winter sown plug plants that were covered with 10 μm BIS mulch film, as previously tested thicker films were economically unviable. Plug plants were produced in single use module trays which were highly flexible. The flexible single use trays were not compatible with the tray carousel system on the planter and as such planting was slowed significantly. Therefore, it was only possible to plant an area 1.5–2 ha rather than 3–4 ha achieved by rhizome planter (Terravesta, pers. comm.). Soil moisture was low at planting and was followed by an extended period of low rain fall and establishment losses were >50%. In these conditions, mulch film has been shown to have a negative effect of germination of direct sown *M. sinensis* seeds (Ashman et al., 2018). Mulch film in these circumstances can inadvertently provide a barrier which can limit the positive effect of rain. An earlier planting date may have allowed the plugs to be established in more favourable conditions with higher soil moisture and higher rainfall after planting. However, the winter sown plugs were not mature enough for planting until early May.

In 2021, the trial was re-run with updated protocols to address the key issues found. In 2020, upscaling to planting 9 ha demonstrated a number of practical issues: (1) module trays that were not compatible with commercial plug planters, (2) a late planting date that could mean low soil moisture content and (3) wide mulch film that could limit the positive effects of rain after planting.

Therefore, in 2021, the protocols were updated to address these issues using (1) a rigid re-usable module tray containing 216 seedlings matched to the machine, (2) an earlier planting date with plug plants sown in late summer and overwintered in a frost-free glasshouse prior to planting and (3) halving the mulch film widths covering 1 row rather than 2 reducing the amount of plastic by 25% and increasing the resilience to wind tearing. Rigid module trays and narrow mulch films increased the planting speeds from 1.5 to 3 ha per day. While the area planted in 2020 and 2021 was small compared to the scale required to meet net zero goals the size of the trials showed that the results are commercially relevant. These trials show that the plug planting protocol developed in 2021 has a planting speed comparable to rhizome planting; however, the logistics required for large-scale deployment of seed-based *Miscanthus* plug plants or the cost point of this establishment method was not assessed. As crop establishment from seed-based plugs plants is widely used for a range of vegetables this agronomic method with existing highly developed logistical chains can now be applied to *Miscanthus*. Although plug planting may be an unfamiliar establishment method to many farmers, there is a wide network of experienced contractors with the necessary specialized machinery and costs will fall as scales increase.

6 | CONCLUSIONS

- For both *Miscanthus* seedlings and rhizomes, spring transplanting and overwintering survival following the first growing season are improved when propagules are covered with transparent mulch film for the first 6–8 weeks after planting.
- Mulch films enhanced establishment success of seed-based plugs more than rhizomes.
- Mulch films increased tillering, stem height and yield up to year 3.
- Higher planting densities increased yields in the early years, but longer-term trials would be needed to evaluate yields and profitability dynamics over the whole crop lifespan.
- Bioderived and biodegradable in soil plastic mulch films produced similar positive results on crop establishment traits to those covered with oxo-degradable films made from petroleum.
- Combining technologies for plug production and planting with narrow bio-based mulch films allowed cost-effective plug production protocols to be developed for commercial upscaling.
- The fiscal barrier of higher *Miscanthus* establishment costs incurred when using bioderived and biodegradable in soil plastic mulch films could be mitigated by either apportionment over the 15–20 year lifespan or an establishment grant or both.
- Bioderived and biodegradable in soil plastic mulch films used to establish *Miscanthus* are an example of the bio-based circular economy.

ACKNOWLEDGEMENTS

This research was supported by the GRACE project funded by the Bio-based Industries 635 Joint Undertaking, grant agreement no. 745012 and UKRI BBSRC funding to the Core Strategic Programme on Resilient Crops (BBS/E/W/0012843A). We acknowledge the contributions of Chris Glover and Robin Warren from the *Miscanthus* Breeding Team at Aberystwyth for collecting the data and managing the field trials. Furthermore, the authors would like to thank Astley Hastings (Aberdeen University) for contributing to the editing of this manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that progress reported in this paper, which includes input from industrial partners, is not biased by their business interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Open Science Framework (OSF) https://osf.io/3tmgj/, DOI: 10.17605/OSF.IO/3TMGJ.

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REFERENCES

Abdelmoez, W., Dahab, I., Ragab, E. M., Abdelsalam, O. A., & Mustafa, A. (2021). Bio-and oxo-degradable plastics: Insights on facts and challenges. *Polymers for Advanced Technologies*, *32*, 1981–1996. https://doi.org/10.1002/pat.5253

Aisbl, P., & Deconinck, S. (2013). Final report benefits and challenges of bio-and oxo-degradable plastics a comparative literature study. *Polymers for Advanced Technologies*, *32*(4), 1–16. https://doi.org/10.1002/pat.5253

Ashman, C., Awty-Carroll, D., Mos, M., Robson, P., & Clifton-Brown, J. (2018). Assessing seed priming, sowing date, and mulch film to improve the germination and survival of direct-sown *Miscanthus sinensis* in the United Kingdom. *GCB Bioenergy*, *10*, 612–627. https://doi.org/10.1111/gcbb.12518

Atkinson, B. S., Sparkes, D. L., & Mooney, S. J. (2007). Using selected soil physical properties of seedbeds to predict crop establishment. *Soil and Tillage Research*, *97*, 218–228. https://doi.org/10.1016/j.still.2007.09.017

Awty-Carroll, D., Magenau, E., Al Hassan, M., Martani, E., Kontek, M., VAN DER Pluijm, P., Ashman, C., De Maupeou, E., Mccalmont, J., Petrie, G.-J., Davey, C., van der Cruijsen, K., Jurišić, V., Amaducci, S., Lamy, I., Shepherd, A., Kam, J., Hoogendam, A., Croci, M., … Clifton-Brown, J. (2023). Yield performance of 14 novel inter- and intra-species *Miscanthus* hybrids across Europe. *GCB Bioenergy*. https://doi.org/10.1111/gcbb.13026

Cantliffe, D. J. (2008). Plug technology: Horticultural revolution. *Chronica*, *48*, 7.

CCC. (2020). *Reducing UK emissions: 2020 progress report to Parliment*.

Christian, D. G., Yates, N. E., & Riche, A. B. (2005). Establishing *Miscanthus sinensis* from seed using conventional sowing methods. *Industrial Crops and Products*, *21*, 109–111. https://doi.org/10.1016/j.indcrop.2004.01.004

Clifton-Brown, J., Harfouche, A., Casler, M. D., Dylan Jones, H., Macalpine, W. J., Murphy-Bokern, D., Smart, L. B., Adler, A., Ashman, C., & Awty-Carroll, D. (2019). Breeding progress and preparedness for mass-scale deployment of perennial lignocellulosic biomass crops switchgrass, miscanthus, willow and poplar. *GCB Bioenergy*, *11*, 118–151. https://doi.org/10.1111/gcbb.12566

Clifton-Brown, J., Hastings, A., Mos, M., Mccalmont, J. P., Ashman, C., Awty-Carroll, D., Cerazy, J., Chiang, Y.-C., Cosentino, S., Cracroft-Eley, W., Scurlock, J., Donnison, I. S., Glover, C., Gołąb, I., Greef, J. M., Gwyn, J., Harding, G., Hayes, C., Helios, W., … Flavell, R. (2017). Progress in upscaling *Miscanthus* biomass production for the European bio-economy with seed-based hybrids. *GCB Bioenergy*, *9*, 6–17. https://doi.org/10.1111/gcbb.12357

Clifton-Brown, J. C., Stampfl, P. F., & Jones, M. B. (2004). *Miscanthus* biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. *Global Change Biology*, *10*, 509–518.

Creutzig, F., Ravindranath, N. H., Berndes, G., Bolwig, S., Bright, R., Cherubini, F., Chum, H., Corbera, E., Delucchi, M., Faaij, A., Fargione, J., Haberl, H., Heath, G., Lucon, O., Plevin, R., Popp, A., Robledo-Abad, C., Rose, S., Smith, P., … Masera, O. (2015). Bioenergy and climate change mitigation: An assessment. *GCB Bioenergy*, *7*, 916–944.

Dauber, J., Cass, S., Gabriel, D., Harte, K., Åström, S., O'rourke, E., & Stout, J. C. (2015). Yield-biodiversity trade-off in patchy fields of *M iscanthus × giganteus*. *GCB Bioenergy*, *7*, 455–467. https://doi.org/10.1111/gcbb.12167

de Mendiburu, F. (2021). *Agricolae tutorial (Version 1.3-5)*. Universidad Nacional Agraria.

Defra. (2021). *Areas of crops grown for bioenergy in England and the UK: 2008–2020*.

Easson, D., & Fearnehough, W. (2000). Effects of plastic mulch, sowing date and cultivar on the yield and maturity of forage maize grown under marginal climatic conditions in Northern Ireland. *Grass and Forage Science*, *55*, 221–231. https://doi.org/10.1046/j.1365-2494.2000.00218.x

Eunomia. (2021). *Conventional and biodegradable plastics in agriculture*.

European Parliament, C. O. T. E. U. (2019). Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment. *Official Journal*, *L55/1*.

Fajardy, M., & Mac Dowell, N. (2017). Can BECCS deliver sustainable and resource efficient negative emissions? *Energy & Environmental Science*, *10*, 1389–1426. https://doi.org/10.1039/C7EE00465F

Farrell, A., & Gilliland, T. (2011). Yield and quality of forage maize grown under marginal climatic conditions in Northern Ireland. *Grass and Forage Science*, *66*, 214–223. https://doi.org/10.1111/j.1365-2494.2010.00778.x

Finch-Savage, W. E., & Bassel, G. W. (2015). Seed vigour and crop establishment: Extending performance beyond adaptation. *Journal of Experimental Botany*, *67*, 567–591.

Fox, J. (2019). *An R companion to applied regression* (3rd ed.). *Sage Publications*.

Götz, M., Rudi, A., Heck, R., Schultmann, F., & Kruse, A. (2022). Processing *Miscanthus* to high-value chemicals: A techno-economic analysis based on process simulation. *GCB Bioenergy*, *14*, 447–462.

Hastings, A., Clifton-Brown, J., Wattenbach, M., Miitchell, C. P., Stampfl, P., & Smith, P. (2009). Future energy potential of *Miscanthus* in Europe. *GCB Bioenergy*, *1*, 180–196.

Hastings, A., Mos, M., Yesufu, J. A., Mccalmont, J., Schwarz, K., Shafei, R., Ashman, C., Nunn, C., Schuele, H., Cosentino, S., Scalici, G., Scordia, D., Wagner, M., & Clifton-Brown, J. (2017). Economic and environmental assessment of seed and rhizome propagated *Miscanthus* in the UK. *Frontiers in Plant Science*, *8*, 1058. https://doi.org/10.3389/fpls.2017.01058

Heaton, E. A., Dohleman, F. G., Miguez, A. F., Juvik, J. A., Lozovaya, V., Widholm, J., Zabotina, O. A., Mcisaac, G. F., David, M. B., & Voigt, T. B. (2010). *Miscanthus*: A promising biomass crop. *Advances in Botanical Research*, *56*, 75–137.

Heaton, E. A., Long, S. P., Voigt, T. B., Jones, M. B., & Clifton-Brown, J. (2004). *Miscanthus* for renewable energy generation: European Union experience and projections for Illinois. *Mitigation and Adaptation Strategies for Global Change*, *9*, 433–451. https://doi.org/10.1023/B:MITI.0000038848.94134.be

Kasirajan, S., & Ngouajio, M. (2012). Polyethylene and biodegradable mulches for agricultural applications: A review. *Agronomy for Sustainable Development*, *32*, 501–529. https://doi.org/10.1007/s13593-011-0068-3

Kwabiah, A. (2005). Growth, maturity, and yield responses of silage maize (*Zea mays* L.) to hybrid, planting date and plastic mulch. *Journal of New Seeds*, *7*, 37–59.

Lask, J., Rukavina, S., Zorić, I., Kam, J., Kiesel, A., Lewandowski, I., & Wagner, M. (2021). Lignocellulosic ethanol production combined with CCS—A study of GHG reductions and potential environmental trade-offs. *GCB Bioenergy*, *13*, 336–347.

Lesur, C., Jeuffroy, M.-H., Makowski, D., Riche, A. B., Shield, I., Yates, N., Fritz, M., Formowitz, B., Grunert, M., & Jorgensen, U. (2013). Modeling long-term yield trends of *Miscanthus × giganteus* using experimental data from across Europe. *Field Crops Research*, *149*, 252–260. https://doi.org/10.1016/j.fcr.2013.05.004

Lewandowski, I., Clifton-Brown, J., Scurlock, J., & Huisman, W. (2000). *Miscanthus*: European experience with a novel energy crop. *Biomass and Bioenergy*, *19*, 209–227.

Lewandowski, I., Scurlock, J. M., Lindvall, E., & Christou, M. (2003). The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, *25*, 335–361.

Li, S., Ding, F., Flury, M., Wang, Z., Xu, L., Li, S., Jones, D. L., & Wang, J. (2022). Macro-and microplastic accumulation in soil after 32 years of plastic film mulching. *Environmental Pollution*, *300*, 118945. https://doi.org/10.1016/j.envpol.2022.118945

Linde-Laursen, I. (1993). Cytogenetic analysis of *Miscanthus* 'Giganteus', an interspecific hybrid. *Hereditas*, *119*, 297–300. https://doi.org/10.1111/j.1601-5223.1993.00297.x

Liu, E., He, W., & Yan, C. (2014). White revolution'to 'white pollution'—Agricultural plastic film mulch in China. *Environmental Research Letters*, *9*, 091001.

Olave, R., Forbes, E., Munoz, F., Laidlaw, A., Easson, D., & Watson, S. (2017). *Performance of Miscanthus × giganteus* (Greef et Deu) established with plastic mulch and grown from a range of rhizomes sizes and densities in a cool temperate climate. *Field Crops Research*, *210*, 81–90.

O'Loughlin, J., Finnan, J., & Mcdonnell, K. (2017). Accelerating early growth in *Miscanthus* with the application of plastic mulch film. *Biomass and Bioenergy*, *100*, 52–61.

Orzolek, M. D. (1991). Establishment of vegetables in the field. *HortTechnology Horttech*, *1*, 78–81.

R Development Core Team. (2015). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

von Hellfeld, R., Hastings, A., Kam, J., Rowe, R., Clifton-Brown, J., Donnison, I., & Shepherd, A. (2022). Expanding the *Miscanthus* market in the UK: Growers in profile and experience, benefits and drawbacks of the bioenergy crop. *GCB Bioenergy*, *14*, 1205–1218.

Witzel, C.-P., & Finger, R. (2016). Economic evaluation of *Miscanthus* production—A review. *Renewable and Sustainable Energy Reviews*, *53*, 681–696. https://doi.org/10.1016/j.rser.2015.08.063

Witzleben, S. (2022). Minimizing the global warming potential with geopolymer-based insulation material with *Miscanthus* fiber. *Polymers*, *14*, 3191.

Xue, S., Kalinina, O., & Lewandowski, I. (2015). Present and future options for *Miscanthus* propagation and establishment. *Renewable and Sustainable Energy Reviews*, *49*, 1233–1246. https://doi.org/10.1016/j.rser.2015.04.168

Yang, Y., Li, Z., Yan, C., Chadwick, D., Jones, D. L., Liu, E., Liu, Q., Bai, R., & He, W. (2022). Kinetics of microplastic generation from different types of mulch films in agricultural soil. *Science of the Total Environment*, *814*, 152572.

Zhang, Q., Zhang, L., Evers, J., Van der Werf, W., Zhang, W., & Duan, L. (2014). Maize yield and quality in response to plant density and application of a novel plant growth regulator. *Field Crops Research*, *164*, 82–89.

Zimmermann, J., Styles, D., Hastings, A., Dauber, J., & Jones, M. B. (2014). Assessing the impact of within crop heterogeneity ('patchiness') in young *Miscanthus × giganteus* fields on economic feasibility and soil carbon sequestration. *GCB Bioenergy*, *6*, 566–576. https://doi.org/10.1111/gcbb.12084

How to cite this article: Ashman, C., Awty-Carroll, D., Mos, M., Kam, J., Guerrini, S., Calder, S., & Clifton-Brown, J. (2023). Developing *Miscanthus* seed plug establishment protocols with mulch film for commercial upscaling. *GCB Bioenergy*, *15*, 746–764. https://doi.org/10.1111/gcbb.13044