Project Final Report
NZIP Biomass Feedstocks Innovation Programme

Project code: NEF153

Title: Technologies to Enhance the Multiplication and Propagation of Energy Crops (TEMPEC)

Contractor: New Energy Farms (NEF)

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1. Executive Summary

Introduction – The overall objective of the TEMPEC project (Technologies to Enhance the Multiplication and Propagation of Energy Crops) was to address the challenge of scaling up the planting of high biomass perennial energy grasses for renewable energy production. UK studies suggest that between 214,000 and 700,000 ha are required, and a current area under 9,000 ha indicates the scale of the task. This project aimed to develop solutions to expand this area, which will not only fulfil the requirement for biomass energy but will also achieve lower Green House Gas (GHG) emissions and increase levels of carbon sequestration. New Energy Farms (NEF) is an agricultural technology licensing company, established to develop crop scale-up and establishment systems for perennial grasses used for fuel, fibre and food. The company has 25 years of experience producing energy crops and has patented a synthetic seed technology, CEEDS™, for application to these crops.

Aims and Objectives – The first was to test new energy grasses from global breeding projects, as most UK energy grass crops are currently planted from one variety. This provides new higher-yielding options for UK growers and reduces the time to introduce these new varieties. The second, to validate if CEEDS™ technology is applicable to UK energy crops and to develop new methods to multiply and establish vegetatively propagated energy crops. Then, to understand the frequency of symbiotic relationships between fungi and bacteria and energy crops in the UK environment and if they are beneficial to the yield of biomass crops. Following this to determine the opportunity for biomass production through paludiculture on wetland reclamation areas and metal-contaminated soils. To determine if NEF's innovative planting technologies can be adapted to wetland (Paludiculture) crops such as *Typha*. Finally, GHG analyses, production models, and licensing strategies for increased biomass production have been developed using the varieties and propagation systems developed in the project.

Results – A diverse range of new energy grasses were imported and tested for the first time in the UK at five sites around the UK, using field testing sites (Figure 1c). Results showed that six varieties could outperform the control of current *Miscanthus* types. With projected peak yields in the range of 16.8 - 29.5 t ha^{-1} yr^{-1} of dry matter based on extrapolation to current field planting densities, compared to typical yields in the range of 10-15 t ha^{-1} yr^{-1}. The trial sites are being continued as other materials imported into the project also show greater potential. Propagation systems were successfully developed based on NEF's CEEDS™ system for a range of high-yielding energy grasses. The work covered all aspects of production from initial propagation through to coating to make the final energy crop CEEDS™ (Figure 1a). Field planters were developed and constructed (Figure 1b). Microbial work completed the most detailed assessment of microbes in UK *Miscanthus* crops, aged 2-27 years of age. The results showed significant differences in fungal populations between sites, but similarity of bacterial populations was not expected and had not been reported before. New propagation and novel planting solutions were developed for planting the paludiculture crop *Typha*. For contaminated land, it was demonstrated how combinations of chemical treatments could support the establishment of *Miscanthus* on land highly contaminated with heavy metals. These outputs were developed into a commercial bio-factory model for CEEDS™ production, an intensive horticultural site that can produce 1,000 ha yr^{-1} of planting material. Using a production footprint 10-20 times lower than conventional field propagation technology combined with fully automatic CEEDS™ planters. Business model licensing was established as the commercialisation route following project completion.

Contribution to Sustainable UK Biomass Supply – This work was based on the projected increase in UK energy crop area to 700,000 ha by 2050. Increases in yield from these new varieties would support greater biomass production, with the same land area capable of producing up to 14 million tonnes of dry matter, compared to current projections of 8.4 million tonnes. Planting these crops from 2026 onwards at a rate of 30,000 ha yr^{-1} would currently require a propagation area of 1,650 ha. This requirement could be reduced tenfold using CEEDS™ bio-factories. Annual planting at this scale using CEEDS™ precision planters would require just 30 planters, compared to 210 manual planters currently needed. Beyond conventional farmland, this project also developed solutions for wetland remediation and previously unsuitable land, unlocking additional feedstock potential while contributing to the remediation of non-productive land.

Impact of Innovation on Greenhouse Gas Emissions – A Life Cycle Analysis was conducted for the propagation systems developed in the project. It showed that GHG emissions could be reduced by 14-18% compared to the baseline emissions associated with establishing *Miscanthus* from rhizomes in the UK.

Figure 1: *Images from the TEMPEC project: (a) Synthetic seed produced using NEF CEEDS™ system; (b) field planter built to drill CEEDS™ into the ground; (c) one of the high yielding energy grass variety testing sites established for the project.*

2. Background

New Energy Farms (NEF) is an agricultural technology licensing company based in Wiltshire, UK. It was established in 2010 to develop crop scale-up and establishment systems. NEF initially focused on developing solutions for plantations of perennial grasses used for fuel, fibre, and food. The management of NEF has over 25 years of extensive experience developing and commercialising vegetative propagation systems for perennial grasses in the EU, North and South America, with first-hand experience in establishing vegetatively multiplied crops at scale. This work led NEF to focus its research activities on developing technologies to establish perennial grasses in a manner similar to row crops.

One of the serious challenges facing the UK is how to scale up the planting of high biomass perennial energy grasses to a level of 30,000 ha each year, to reach even the lowest proposed targets of 214,000 ha. The current total planted area of about 8,800 ha of *Miscanthus* in the UK[1] clearly indicate the scale of the task. This project aimed to meet this target with solutions which would not only fulfil the requirement for biomass for energy but will also achieve lower greenhouse gas (GHG) emissions and increase levels of carbon sequestration.

Currently, the majority of energy grass crops planted in the UK consist of a single *Miscanthus* variety. Any expansion of the planted area must include a broader range of varieties to ensure greater genetic diversity and long-term cropping stability. The small scale of the current market has not incentivised expansion of the variety base available to growers. NEF has been field testing a limited number of new *Miscanthus* varieties from two non-UK breeding programmes over several seasons. However, there are other international breeding programmes that may be producing high-biomass grasses suitable for UK conditions, which have yet to be evaluated in UK environments. Expanding the number of varieties available to growers is common in all cropping systems as production areas grow, providing increased resilience through genetic diversity. Sourcing new varieties from existing, well-established breeding programmes can also reduce the timeline for variety introduction—from nearly ten years down to as little as three.

Crops that are not yet produced on a large scale – such as high-biomass energy grasses in the UK – currently attract limited investment in the improvement of production techniques. A further constraint on expansion is that many high-biomass energy crops do not produce seeds and must instead be vegetatively propagated – a multiplication method that is significantly more costly than seed production. To address this, NEF has developed a patented technology called CEEDS™ (Crop Expansion, Encapsulation and Drilling System), which is currently being adopted in sugarcane production. This system helps overcome the challenges and costs associated with multiplying and planting vegetatively propagated crops. Furthermore, transferring technologies into different and often unrelated market sectors can reveal new opportunities to improve production systems and increase yields. CEEDS™ also serves as a field delivery method that can incorporate beneficial biological products into the planted propagule, further enhancing energy grass performance.

The combination of new, higher-yielding varieties and improved establishment methods – two strategies that have proven successful in many cropping systems globally – could act as key

[1] https://www.gov.uk/government/statistics/bioenergy-crops-in-england-and-the-uk-2008-2023

catalysts for scaling up energy crop production, delivering higher yields per hectare and significantly increasing biomass output.

CEEDS™ may have applications beyond food and energy crops grown on arable land. Large areas of land considered borderline for the economical production of food crops could, in fact, be well suited for energy crop cultivation. Biomass can be produced on low-quality arable land; phytoremediation can be practiced on metal-contaminated land (spoil land); and degraded wetlands – significant sources of GHG emissions – can also support the growth of high-biomass grasses. To unlock their full potential, novel technologies like CEEDS™ should not be limited to conventional production systems, but explored across a broader range of land types and environmental challenges.

3. Project Overview

3.1 Aims and Objectives

There were five objectives for the projects aligned with the five work deliverables, these were:

Varieties – Evaluate *Miscanthus* varieties from worldwide breeding programmes and other high biomass genera (particularly from the *Saccharum* complex) from breeding programmes that are producing material potentially suitable for UK and EU conditions. The objective was to increase the yield by importing new varieties of energy grasses to the UK. Over 95% of UK *Miscanthus* is *Miscanthus* x giganteus. Energy crop breeding programmes around the world are virtually untapped by UK growers. The UK cannot ignore the opportunities that already exist to evaluate novel genera, species and existing (but new to the UK) varieties to improve yield, expand production areas and widen the genetic base. As a result, 27 varieties were trialled. The selection of varieties for importation was largely determined by their reported cold tolerance to ensure that they were likely to have some suitability for UK environments, so NEF targeted 'regions of origin' where Hardiness Zones overlapped with comparable Hardiness Zones (whilst ensuring different Hardiness Zone systems were aligned) to those in the UK. All imported material also had to have recorded yield and performance data to be eligible for entry into the trials.

Propagation – To validate if CEEDS™, technology is applicable to UK high biomass crops. To develop new methods to multiply and establish vegetatively propagated energy crops. NEF has worked in vegetative propagation of energy grasses for 25 years, including first-generation UK *Miscanthus* rhizome propagation. This component was based on developing two technologies from NEF, CEEDS™ an artificial seed system, and priming a 2nd generation *Miscanthus* rhizome system.

Microbial Interaction – The objective was to understand the frequency of symbiotic relationships between fungi/bacteria and energy crops in the UK environment. Could the synergies between crops and endophytes or ectophytes, or other beneficial fungi and bacteria, be captured in energy grasses and developed to benefit biomass crops? Members of *Poaceae* (grasses) are highly responsive to microbial interactions – a generic term favoured to describe these organisms. However, these associations appear to be highly dependent on climate and genus. Microbial associations in commercial UK *Miscanthus* crops, some dating back to planting dates of 1998, were explored to identify possible beneficial (or pathogenic) associations.

Marginal Land (Wetlands and Contaminated Land) - Determine the opportunities for biomass production through paludiculture on wetland reclamation areas and metal contaminated soils. *The England Peat Action Plan and UK Net Zero* Strategy plan[2] intends to restore 280,000 ha of UK wetlands by 2050. Reducing the high emission levels that are released from drained and eroded peat areas is also a major target in many countries. This objective was focused on NEF's innovative planting technologies to determine if they could be adapted to paludiculture crops such as *Phragmites, Typha, Molinia* or *Phalaris*. It was also important to determine if these biomass plants could be scaled to sequester carbon, restore wildlife and used to extract phosphate from wetlands. Just under 2% of UK land area is classed as contaminated, with 60% being metal contamination. Spoil heaps from mining activities and other degraded soils cover considerable areas and currently there are few methods of large-scale remediation. CEEDS™ technology plus energy crops could be one useful phytoremediation tool.

Commercial Implementation and Carbon Mitigation – To develop GHG analyses, production models and licensing strategies for increased biomass production using the varieties and propagation systems developed in the project.

Schedule, Deliverables and Financial

Scheduled to start in March 2022, commencement delays meant that the project did not get underway until May 2022. The schedule of work was split over a three-year period and divided into five key Work Packages (WP) namely:

- Import and test new energy grasses (WP 1).
- Develop CEEDS™ and priming propagation systems (WP 2).
- Improve energy grass performance with microbials (WP 3).
- Extend biomass production to wetlands and contaminated soils (WP 4).
- Market Analysis and Commercial Structures (WP 5).

Each Work Package was divided into several deliverables critical to the research and validation of the trials. With a key trial site and research staff based out of a farm site in Taunton, specimen trials were run across a total of five different sites within the UK to explore different growing conditions.

Budgets were allocated according to the deliverables in each of the Work Packages with a clearly defined start and end date and included Labour and Overheads, Capex items, Subcontractors (including Warwick and Plymouth University), Materials, Travel and Subsistence.

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[2] https://assets.publishing.service.gov.uk/media/6116353fe90e07054eb85d8b/england-peat-action-plan.pdf

The project was completed at the end of May 2025 with a total spend on a budget of £2,400,179.46 (excluding VAT).

3.2 Design and Development of the Innovation

The four technical areas were assessed at the start and end of the project for their TRL levels.

The variety assessment started at TRL 6, because it was built on the individual breeders' knowledge of which of their own varieties could be considered suitable for trialling plus the NEF requirement that suitable cold tolerance should be considered. This tried to ensure that 'probable' not 'possible' cultivars were imported. Material Access Agreements were secured with six breeding organisations, covering *Miscanthus,* Miscane (*Miscanthus* x sugarcane cross), Energy cane (cold tolerant sugarcane), [redacted].

All material had to be imported as tissue cultured plants which incurred considerable expense, and technical challenges, before any material could be planted in the field. Initially, up to 40 varieties were planned to be available in spring 2022 but changes in Phytosanitary regulations did not allow the importation from Canada of 11 *Miscanthus* varieties. A total of four *Miscanthus* varieties (from two breeders) also failed during two attempts at tissue culturing and were not available from the breeders.

At the end of the project this component was assessed at TRL 8 as whilst the number of candidate varieties was lower than planned (30), those that did survive tissue culturing and transportation were all available for field testing. Considering that 95% of UK plantings are one variety of *Miscanthus* x giganteus this is a very significant increase in potential variety availability available to growers.

Propagation of UK energy grasses using CEEDS™ technology was assessed at the start of the project, at TRL 5, with priming technology also assessed at TRL 5. At the end of the project both components were assessed as TRL 6.

Work on assessing microbials was assessed at the start of the project, as TRL 5. This assessment was largely based on the knowledge that microbial associations are being identified in many native and cultivated plant situations and one specific research project in New Zealand, reported in 2016, found a beneficial association with one specific fungus and *Miscanthus*. At the end of the project this component was assessed at TRL 7 due to the detailed information from the extensive survey of *Miscanthus* crop associations and the ability to test potential associations in *Miscanthus* crops in the field and in wetland and contaminated soils, with enhanced establishment levels recorded from some treatments.

The Technical Readiness of the Paludiculture studies and the trial work on contaminated land was initially assessed as TRL 4. This was due to the diversity of species involved, limited prior work on propagation of paludiculture grasses, and the recognition that phytoremediation on contaminated soils can be a prolonged process. By the end of the project, this component had progressed to TRL 7. A promising planting propagule was developed for the primary paludiculture target genus, *Typha*, and encouraging establishment rates were recorded in contaminated soil experiments across pot trials and field trials, using a range of mixed additives. Additionally, the project successfully demonstrated the feasibility of delivering planting propagules via drone-based systems.

4. Results

Results are presented here for the five Work Packages (WP), additional information is referenced to the enclosed Annexes for each WP.

4.1 Import and Test New Energy Grasses

Additional figures referenced here are provided in Annex 1.

4.1.1 Introduction

High biomass grasses feature as strong candidates to increase the supply of renewable biomass in the strategic projections of Governments in the UK, Europe and the US. However, the current supply chain for renewable energy from biomass is dominated by wood. High biomass grasses currently have a less important supply role, but this could dramatically change if more high yielding grasses can be identified and enter commercial production. Over 95% of *Miscanthus* currently being grown in the UK for renewable energy is one variety, *Miscanthus* x giganteus. A NEF review of yield performance of long-term UK *Miscanthus* crops over ten years from farm to trial levels produced a yield range of between 9.87t / ha yr to 15 t ha / yr. The only UK investment into a *Miscanthus* breeding programme has been at the Institute of Biological, Environmental & Rural Sciences (IBERS). High biomass grass breeding programmes around the world are virtually untapped by UK growers. The UK renewable industry has yet to fully explore the opportunities which already exist to evaluate novel genera, species and existing (but new to the UK) varieties to improve yield, expand production areas and widen the genetic base. A review for this project of energy crop breeding programmes worldwide highlighted several programmes that could potentially contain ‘field ready’ material which might be suitable for the UK. As it takes at least 10-15 years to breed new perennial grasses, introduction of these new types could reduce that timeframe to three years, which would be three to five times faster.

Four additional high biomass grasses were identified as candidates to consider for the UK market.

Miscane – An interesting hybrid that has been created by crossing sugarcane varieties with varieties of *Miscanthus*. Originally developed to transfer disease resistance genes from *Miscanthus* into sugarcane, it was later discovered that these hybrids also inherited cold tolerance from their *Miscanthus* parent. This made it feasible to grow them further north in the

USA as high-biomass crops. The question posed within this project was whether these hybrids possess sufficient cold tolerance to be viable in the UK environment.

Energy Cane – Energy canes are types of sugarcane cultivars that have been specifically developed for high-fibre, lower sucrose concentrations and increased cold tolerance. They are targeted for high biomass rather than sugar production. Energy canes can produce high yields of lignocellulosic biomass in favourable environments and have greater potential climate ranges than current commercial sugarcane cultivars, making them strong candidates for cellulosic ethanol production. However, the energy canes can be adversely affected by cold winter temperatures. Freeze events in winter and late spring can detrimentally effect energy canes, reducing their yield in a particular season or even killing the plants. It is interesting to note that many experimental programmes in the USA test energy cane varieties over a period of seven years to address this cold tolerance issue.

[redacted], and more recently as a feedstock for anaerobic digestion. Active breeding programmes exist across these regions, and the material used in this project was sourced from one such breeder.

[redacted]

Material Access Agreements, which allow third-party field testing of unregistered genetic material, were secured with six breeding organisations. These agreements covered the four grass types described above, along with additional *Miscanthus* varieties. All targeted material belonged to the *Saccharum* complex mentioned earlier – highlighting the global focus on this botanical family as a key source of alternative high-biomass plant options. A total of 40 varieties were identified as potentially available in spring 2022, subject to successful access agreements and, in many cases, successful importation into the UK. At the start of the project, only seven candidate varieties were available. Expanding the range of both genera and varieties within genera is expected to improve yields and broaden the geographic areas across the UK where high-biomass grasses can be successfully grown.

The purpose was not to create another 15-year energy grass breeding project. It was to explore, by field evaluation, the potential to enhance UK biomass feedstock production by harnessing the existing output from a number of global breeding projects that already had 10-15 years of breeding work completed and had genetic material (varieties) which were already available for field evaluation.

4.1.2 Variety Importation

Phytosanitary regulations do not allow the importation of *Poaeace* into the UK unless they enter an extended period of quarantine (which could be over 12 months) or they are imported as tissue cultured (TC) plantlets, so the TC plantlet route was chosen to import all material.

Tissue culturing plants is a skilled operation and due to the delicate nature of the plant tissue, two problems can arise. TC plant material, which starts as single or small numbers of cells being cultured typically on an agar medium in a sterilised flask, may be so delicate that the plants do not survive transportation procedures which can involve significant package handling procedures and temperature variations. Fungal and bacterial infections are also very common in TC work and can usually be eradicated if treated at the first sign of infection. However, when the material enters a transportation cycle, sometimes up to six days, and it is not treated, it can kill the infected plants.

All incoming material was shipped directly from the airport of arrival in the UK to a Tissue Culture Laboratory in the UK, an arrangement created by NEF, to ensure the best recovery from transportation route for the material. Once the material was suitable for potting on it was transported to the NEF site at Taunton and grown on in polytunnels until field ready. A total of six consignments of tissue cultured plants were undertaken during the importation of material for the Project. The importations spanned a period of 23 months, and the courier company was changed part way through this importation period due to some transport delays.

Plant varieties were sourced from five breeding programmes. Breeders often assign complex alphanumeric codes to their individual lines from a breeding programme. Rather than use these complex codes NEF elected to use a simpler code for each variety. This also did not disclose the codes to any third party, a request made by some of the breeders.

[redacted] – Two genera were made available from the breeding programmes at this university, the material being tissued cultured in their in-house laboratory. Four varieties of *Miscanthus* were dispatched in the first consignment, September 2021, but only three varieties survived. A second, back up consignment of the three varieties took place in March 2023 but unfortunately there was no further tissue cultured material of the fourth variety available. The varieties available for testing were coded M3, M5 and M6. [redacted] lines were also provided as it has recently been targeted for breeding as a bioenergy plant. This is the first field testing of [redacted]. The varieties available for testing were coded T40 and T41. The two varieties, initially imported in August 2021, failed to survive the transportation and further material was tissue cultured and sent in 2022. These plantlets survived transportation but were exceptionally slow to develop after being tissue cultured and were not ready to be planted in the field until 2023. Once planted they were very slow to grow during 2023 but at the end of 2024, they were starting to suggest that they had the potential to develop into large plants.

[redacted] – The six variety lines of *Miscanthus*, nominated by this university, were tissue cultured in a research service agreement and shipped to the UK in January 2022. They were delivered to the tissue culture lab in England in February, within days. This material had been dispatched in small, 50ml conical tubes with only between two and eight plantlets of each line. Unfortunately, two of the cultures were 'bleeding', the term used to describe bacterial movement from the plant tissue into the gel medium in the test tube. These two varieties died from the contamination and a third variety just failed to grow from the initial plantlet stage and died in April 2022. NEF approached the tissue culture laboratory to seek replacements but a quotation for 140 plantlets was considered to be too high. The three varieties that were therefore available for testing were coded T13, T14 and T15.

[redacted] – A research services agreement was made with this university in March 2022 to produce, using tissue culture techniques, 25 to 30 explants from each of four [redacted] represents another genus within the family P*oaceae*.

The research services agreement included four selected [redacted] accessions. The first dispatch of plantlets of 72 plantlets was during August 2022 and arrived in the UK in August. A second shipment of 88 plantlets arrived during September 2022. One of the candidate varieties was contaminated upon arrival and, unfortunately, did not survive. The varieties available for testing were coded P70, P71, P72, and [redacted] - a commercial variety used as a control.

[redacted]– The six variety lines - three each of Energy cane and Miscane, were tissue cultured at the in-house facility at this university and dispatched to the UK in February 2022. Unfortunately, there were delays in transit, and the plantlets were not delivered to the nominated UK tissue culture laboratory, until March 2022.Fortunately, the excellent quality of the material supplied by the laboratory, ensured that no variety was lost because of the extended transit time. Energycanes, the sugarcane hybrids that have been developed for more cold tolerance, higher biomass and less sugar content than sugarcane were coded EC50, EC51, EC52. Miscanes, the hybrids produced from sugarcane x *Miscanthus* crosses and targeted for higher biomass production were coded MC60, MC61 and MC62.

Mendel Biotechnology Inc. – The Mendel *Miscanthus* breeding programme originated from them purchasing the Tinplant breeding programme in 2007, which was based in Germany. NEF already had access to four *Miscanthus* lines from this breeding programme so Nagara, and three other varieties which were coded M7, M8 and M9, were included in the trials programme.

NEF also had access to three other *Miscanthus* x giganteus types. Clone Illinois, an original selection from *Miscanthus*, and currently the main variety grown in the USA, and two varieties from Eastern Europe which were coded M27 and M28. The UK *Miscanthus* breeding programme at IBERS were unable to supply any material for variety testing due to an existing commercial agreement with another company in the UK.

The importation period lasted from early 2021 through to March 2023. Once all material had been transferred to the UK tissue culture facility for 'recovery', and then to the Taunton base for growing on, there were considerable differences in the size and age of the plants from the different breeder sources. It was therefore necessary to develop a plan to group the plants not just into different genera (which were growing at different rates) but also into different target planting dates, so that only material of the same age and size was being evaluated in the same trials.

The original aim in the project was to plant the first specimen trials in late spring 2022 but the tissue culture programmes for much of the material being imported had proved to be longer than initially promised and, in some cases, had not provided as many plants as initially indicated. Rather than wait until all the material was uniformly ready for the first field trial it was decided to implement a series of planting dates which commenced in 2022, and went until mid-2023, but always with the control variety present to produce the correct performance comparisons.

4.1.3 Planting Specimen Trials at Five Locations in England

Prior to the project, NEF had discussions with plant breeders, across Europe and the other regions, to ensure that the field-testing proposals for specimen plots and replicated yield trials met with universal approval within the community of breeders in companies and universities that are involved in breeding high biomass grasses. Specimen plots were the favoured first stage of variety testing. Specimen plots contain single plants from each candidate variety plus, for comparison, an individual plant of a control variety. Each plot contained nine candidate varieties and a control variety, and each individual variety was planted in three separate (replicate) plots at each site. The format chosen for specimen trials was a 9m long x 4m wide plot with the plant spacing a 2m x 2m square format for the ten plants in each plot. The spacing of plants in these plots does not reproduce the environment of a commercial field of plants but it does allow very accurate recording of each individual variety's characteristics. Some estimates on relative yields can also be determined.

Figure 2: *Locations of specimen plot trials in the UK for the project.*

Five locations in England were selected to give a good geographic and climatic spread (Figure 2). They also represented, in the cases of Wadebridge, Taunton and Horncastle, as areas in the UK with the greatest planted areas of *Miscanthus*. The Isle of Wight and Darlington represented areas which currently have lower numbers of commercial plantings of *Miscanthus*.

These geographic differences in planting locations were also considered to be important to give easier access, in more areas of the country, to growers who wished to attend open days that were organised at each site during the three years of the project. At each location NEF had a land access agreement with the host farmer to compensate for lost planting area and the 'inconvenience' of an area within a field that normal field operations had to avoid. Throughout the whole project the support and enthusiasm shown by the host farmers was excellent and is therefore acknowledged in this report.

The planting dates at the specimen trial locations are shown in Table 1, beginning in May 2021. Prior to the commencement of the project, the specimen plot trial format was field-tested at the Taunton location, using nine candidate varieties and one control variety already available to NEF. As additional candidate varieties became available in 2022 and 2023, they were planted at the specimen plot locations alongside controls. The two planting dates in 2023 reflect the fact that some variety material was not yet suitable for outdoor planting on the first available date and was therefore delayed until ready.

Table 1: *Specimen plot planting dates at the five trial locations in the UK.*

Location	2021	2022	2023 (1st)	2023 (2nd)
Taunton	14th May	29th June	2nd May	14th June
Wadebridge	*	7th July	16th May	19th June
Shalcombe (Isle of Wight)	*	13th July	17th May	20th June
Horncastle	*	21st July	26th May	13th July
Darlington	*	*	*	5th June

During 2023, data were collected from the varieties in the specimen trials, as outlined below:

- **Cold tolerance:** A critical assessment for any new varietal material introduced into the UK.
- **Stem thickness:** Measured at harvest; this data can inform potential end-use applications, particularly in areas such as animal bedding.
- **Stem number:** Important for comparing how different genera and varieties respond to climatic conditions and planting densities.
- **Plant height:** Recorded at the onset of senescence.
- **Yield data:** Collected in late spring each year from all five specimen plot locations. Yield data from perennial crops must be interpreted with caution in the early seasons after establishment. It is widely recognised in the biomass industry that large perennial biomass grasses require several seasons to reach their plateau yields – levels that remain relatively stable, aside from minor annual fluctuations due to variations in rainfall and accumulated heat units.

Commercially, many high-biomass crops are not harvested in their first or second year, as early yields often do not justify the cost of harvesting. However, when evaluating new genera and varieties, it is important to monitor yield progression, as some may exhibit different establishment and productivity patterns. For this reason, every variety in each specimen plot at every site was individually harvested each year.

High-biomass grasses are typically not harvested in autumn, as they require a prolonged senescence period during which the leaves and stems dry down. Instead, harvest takes place in March or April, just before new growth appears. For example, the first harvest date for a 2021-planted specimen trial occurred in March 2022 (Table 2). The only exception to this timing was the [redacted] varieties, which were harvested in autumn due to their known intolerance to low winter temperatures; if left in the field, their tissues would begin to break down and decompose over the winter months.

Table 2: *Specimen plot harvest dates at the five UK trial locations.*

Location	2022	2023	2024	2025
Taunton	_____?_	13th April	28th February	25th March
Wadebridge	*	31st March	10th March	18th March
Shalcombe IOW	*	27th March	28th March	25th March
Horncastle	*	17th April	19th March	14th April
Darlington	*	*	10th April	1st April

Cold Tolerance

During the short, three-year duration of this project, the UK experienced several notable weather events that broke existing records. For example, 2022 was the warmest year on record for both maximum and mean temperatures in the UK, based on data dating back to 1884. This was followed by 2023, the second warmest year, and then the summer of 2024, which was the Earth's warmest on record – although, in contrast, the UK experienced its coolest summer since 2015. None of these higher temperature events were considered problematic for the high-biomass grasses under evaluation. Typically, it is low temperatures that determine plant survival, as these perennial species must possess sufficient cold tolerance to endure winter conditions and regrow in the spring.

Weather data obtained from the specimen trial locations was useful for interpreting the physiological and yield data between 2022 and 2025. The lowest recorded temperature at Taunton during the winter of 2023/24 occurred in January, reaching -6.5°C. The other four locations recorded their lowest temperatures in February 2024: Horncastle: -5.0°C, Wadebridge: -5.0°C, Isle of Wight: -6.0°C, and Darlington: -6.0°C. 2023/24 was also one of the wettest winters on record. In contrast, the winter of 2024/25 had 11% less rainfall than the long-term average, with above-average temperatures in both December and February. The average temperature for the season was 4.6°C, which is 0.5°C above the long-term meteorological average. The lowest temperatures at each trial location during this winter were:

Taunton: - 5^0C in November 2024
Wadebridge: -3^0C in January 2025
Shalcombe (Isle of Wight): -6^0C in January 2025
Darlington: -6^0C in November 2024 and January 2025
Horncastle: -7^0C in November 2024 and January 2025

The three Miscane varieties and the Energy cane varieties did not survive their first winters in the UK. These were subsequently replaced in the individual plots with new plants of the same variety. While it is important to record that these varieties failed to survive their first winter, it is also important to note that the replacement plants survived the following winter of 2024/25. This point is discussed in the relevant genera sections.

NEF staff have been working with *Miscanthus* x giganteus for well over 20 years, Clone Illinois being a 15-year experience, but all exhibit very similar cold tolerances which allows them to be successfully grown in the UK with no concerns about not surviving UK winters. The variety with the most tolerance in the Project assessments appears to be *Miscanthus* M7, a variety from the European breeding programme.

The two [redacted] varieties have lower cold tolerance than any of the *Miscanthus* varieties in this assessment and one of the energy canes is also indicating cold intolerance. The three Miscanes appear to have cold tolerances similar to those of the best *Miscanthus* varieties.

Lack of cold tolerance in the autumn does not automatically mean that plants will not survive the winter. [redacted] varieties have low cold tolerance and began senescing when they experience the first low temperatures in the autumn. This reaction triggered the decision to conduct an early harvest (Autumn) on the four varieties. However, they appear to have insufficient cold tolerance to senesce during a UK autumn and be harvested over winter as dry biomass, but the majority survived UK winters in southern sites.

4.1.4 **Results:** *Miscanthus* Varieties

The harvesting methodology for high biomass grasses, employed by the majority of breeding companies, was used by NEF. It involves cutting the plants at 10cm above soil level and then weighing the individual plant biomass to obtain a fresh weight. The material is then oven dried to allow a dry weight assessment to be calculated and presented. The yield data from a specimen plot will not necessarily reflect how a variety may perform in a commercial field situation, but it does give an initial indication of possible yield differences between varieties. As described earlier, the different genera and varieties became available for field trials at different times so in 2021 only seven *Miscanthus* varieties were in specimen trials but by 2023 all 14 had been planted. Using the control variety *Miscanthus* x giganteus, the performance of the candidate varieties could always be compared to a constant control performance.

The Taunton site had the longest harvest record with some material having four harvests. The first planting was in 2021, yielding a 2022 harvest - the first of four harvests for some varieties. However, some sites had only two harvests (2024 and 2025), due to delayed planting until 2023 as a result of material availability.

Four sites were planted with specimen plots in 2022: Taunton, Wadebridge, Isle of Wight, and Horncastle. In 2023, additional varieties were planted at each of these sites, and at a fifth site, Darlington, bringing the total number of sites to five (see Figure 2).

The yield plateau mentioned earlier can be identified in the *Miscanthus* yield data from varieties that had four years of harvests (Figure 12). Seven varieties reached this four-harvest threshold and are demonstrating the plateauing effect, while the others – having had only two harvests – are still on an upward yield trajectory. Yield data are expressed as individual plant dry weights in the specimen trials. Harvest results from each individual site are also presented.

At Taunton, the varieties M7, Nagara and M27 are the highest yielding (Figure 13), and then there is a considerable lowering in yield before those of M28, M8 and M9. The highest yielding variety at Horncastle was M27, closely followed by a group containing M7, M8, M28 and Nagara, (Figure 14). The varieties M7 and M28 were the highest yielders at Wadebridge, but M27, Nagara and M9 also performed well (Figure 15). Darlington was the second lowest yielding site, but it was the latest site to be planted. Nagara was the highest yielding variety along with M28 and M7, (Figure 16). M9, M8 and M27 were the three next highest yielding varieties. The lowest yielding site was the Isle of Wight, but it was an exposed site with westerly winds very common. The highest yielding variety after two seasons was M27 (Figure 17), but M28 did not perform well at this location.

The average total dry weight of each *Miscanthus* variety harvested from each location is presented in Figure 18. Single plant yields, although calculated from three replicated separate plots, do not precisely reflect current commercial field planting densities. However, they can give interesting pointers to the possible relative performances of different varieties. Unfortunately, the varieties M3, M5 and M6 were not represented at every specimen plot site due to a shortage of plants. Each single plant, at each of the five specimen trial sites, was individually weighed, to create the data set reported here. Horncastle was the highest yielding site overall with Wadebridge second. The overall highest yielding variety was M7 followed by M27, Nagara, M28, M8 and M9. The comparative performances from the second harvest year, averaged over the five specimen plot sites clearly highlights the excellent performance of the six varieties from the European breeding programmes (Figure 19).

One interesting observation during harvesting, which involves cutting stems at 10cm from soil level, is that it focused attention on the stem structure at the base of the plant. It was very noticeable that one group of varieties, M13, M14 and M15, all from the same breeding programme, still had quite green tissue inside the cut bases of the stems suggesting they were not fully senesced. As harvest time was correctly timed, for current UK grown *Miscanthus* varieties and leaf material had senesced and fallen, this suggests that the three varieties may not fully senesce, in a typical UK winter (Figure 20). The average dry weights of all the varieties illustrate that eleven varieties had average dry weights of 70% to 83% at harvest. However, the other three varieties, M13, M14 and M15 had much lower dry weights of 54% to 58%. Retaining moisture in stems is perhaps not a desirable feature as it can complicate drying procedures for the cut cane, increasing the drying costs and potentially creating overheating problems in storage.

4.1.5 **Results:** Energy Cane

This material has been specifically bred to extend sugarcane production areas into cooler regions, so it is not competing with sugar/ethanol production but is creating biomass for other end uses. The three varieties obtained from the breeding programme were first planted in 2023 and several plants, of all three varieties, did not survive their first UK winter. Replanting in the same positions in the specimen plots took place in late spring of 2024. It was clear from the first cold tolerance ratings, collected in late 2023 (Table 3), that energy canes were expressing their inability to withstand cold temperatures. On a scale from nine (green tissue, with no evidence of cold stress) to two (tissue turning brown and visibly damaged by cold) the energy cane varieties quickly lost their green tissue in late November and gave every indication of not surviving winter by the December assessments.

Table 3: *Cold tolerance ratings, Energycane, 2023.*

Variety	15 November	24 November	1 December
***Miscanthus* x giganteus**	8	6	4
***Miscanthus* Nagara**	8	6	4
EC50	3	3	2
EC51	7	5	3
EC52	7	5	3

Not all the Energy cane plants died in the specimen trials, but surviving plants were very small. The yield progression of the three energy canes, compared to *Miscanthus* x giganteus control performance (Figure 21) demonstrate that only one variety, EC51, appeared to have any useful level of cold tolerance.

EC51 was clearly the most suited to the UK environment of the three Energy canes. The dry weights of the three Energy canes from the second harvest (Spring 2025) are presented in (Figure 22). One factor that must be considered when assessing the performance of these energy canes, apart from their obvious low cold tolerance backgrounds coming from their sugarcane parentage, is that they were initially tissue cultured to allow transfer to the UK. There is a possibility that the process of tissue culturing material may create physiological reasons as to why some plants perform in certain ways. It is that uncertainty that has led to the replanting of trials even though the initial evidence suggests that they are unsuited to the UK environment. The performance of the material in future years will continue to be monitored.

4.1.6 **Results:** Miscane

These *Miscanthus* x sugarcane crosses are primarily exploring whether the biomass potential of sugarcane can enhance the yield of *Miscanthus* by the creation of hybrids. Three varieties of Miscane were imported, via tissue cultured plants, and first planted in specimen trials in 2023. The Miscane varieties were present at all five specimen plot trial sites. Miscanes appear to be more cold-tolerant than energy canes, but their cold tolerance assessments suggested that they may still struggle to survive UK winter conditions (Table 4). The growth of the varieties in their first year, 2023, was very slow and the yield data that was collected indicated plant survival rather than growth (Figure 23). Approaching the 2024/25 winter the plants were still very small, just one tenth the size of *Miscanthus* control plants in terms of dry weight, when they were harvested in March/April 2025. All three Miscane varieties, even at the second harvest, produced very low yields. Surprisingly, they were lower yielding than the Energy canes (Figure 24). In the 2024 season the Miscane plants had clearly established and were growing but once again the growth rate was very slow. The dry weights of the plants, when harvested in March /April 2025, were very low; the average per plant across all the sites being 120g to 160gm compared to the *Miscanthus* control of 620 gm.

Table 4: *Cold tolerance rating of Miscane, 2023.*

Variety	15 November	24 November	1 December
***Miscanthus* x giganteus**	8	6	4
***Miscanthus* Nagara**	8	6	4
MC60	8	8	4
MC61	9	8	4
MC62	9	9	5

4.1.7 Results: [redacted]

This genus is another high biomass grass and varieties are being specifically bred to improve cold tolerance so that they can be successfully grown [redacted] than is currently possible. The mature grass looks like [redacted] and, in its native environment, can grow to 4m tall. Three varieties were obtained from a breeding programme and transported to the UK as tissue cultured plants. The plantlets were very slow to grow compared to the growth of tissue culture plantlets of *Miscanthus*, Energy cane and Miscane and they were only just suitable for planting in spring 2023 as they were very small but appeared sturdy.

Table 5: *Cold tolerance rating of* [redacted]*, 2023.*

Variety	15 November	24 November	1 December
***Miscanthus* x giganteus**	8	6	4
***Miscanthus* Nagara**	8	6	4
T40	6	5	2
T41	4	5	2

After the first year in the field the plants were hardly any larger than when they had been planted and there was some doubt as to whether they had survived their first UK winter as they had cold tolerance ratings similar to those of energy canes, slightly lower than those of the Miscanes, (Table 5). They did survive the first winter of 2023/4, but the plants were very small and did not look like high biomass grasses. The two-year dry weight graph, Figure 25, clearly indicates how small the plants were at their two harvest dates of 2023/4 and 2024/5. Approaching the 2024/25 winter the plants were still very small. Note the data below where they were one tenth the size of *Miscanthus* control plants, in terms of dry weight, when they were harvested in March/April 2025. The indications were that this material was unsuited to the UK. The average plant weight across the five sites was 40gm for T40 and 50gm for T41 compared to the *Miscanthus* control of 620gm, (Figure 26). This poor yield performance, coupled with such a long establishment 'lead time' suggests that they could hardly be considered a commercial high biomass option. Approaching the 2024/25 winter the plants were still very small. Note the data in Figure 26 where they were one tenth the size of *Miscanthus* control plants, in terms of dry weight, when they were harvested in March/April 2025. However, in spring 2025 the [redacted] plants started to grow very rapidly and look capable of producing significantly higher biomass per plant than in previous years. In late April 2025 the plants were larger than those harvested in the 2025 harvest in early April 2025 (Figure 38).

4.1.8 Results: [redacted]

This genus, commonly referred to as [redacted]. The first dispatch of plantlets to the UK, containing 72 tissue cultured plantlets was on 18th August 2022 and arrived at the UK destination, on 22nd August. A second shipment on September 15, 2022, contained a further 88 plantlets. As the plantlets did not arrive in the UK until late summer they were too late for the 2022 planting and were planted in 2023. The four [redacted] varieties were planted at the five specimen trial locations. There was insufficient plant material to enter them into the 2023 Replicated yield plots. One of the varieties, [redacted], the control variety, was first registered in 1989. The other three varieties were coded P70, P71 and P72. The growth rates in the first season were quite spectacular, Figure 27 and Figure 28. The fresh weight yields of the four [redacted], when harvested in the autumn of 2023, were very impressive after such a short growing season (Table 6). Three control *Miscanthus* varieties were also harvested at the same time even though they would not normally be harvested in the autumn, but as dry tissue in early spring.

***Table 6**: [redacted] harvest weights (kg/plant fresh weight), 2023.*

Variety	Taunton	Isle of Wight	Wadebridge	Horncastle	Darlington
***Miscanthus* x giganteus**	0.51	0.15	0.48	0.28	0.49
***Miscanthus* Nagara**	0.62	0.76	0.99	0.48	
Nagara	0.71	0.70	0.13	0.78	0.35
[redacted]	11.40	0.35	1.40	7.54	1.23
P70	10.39	0.35	1.21	4.01	2.00
P71	10.83	0.62	2.50	8.62	2.03
P72	12.34	0.52	1.24	4.13	0.79
Harvest Date	28/11/2023	04/12/2023	22/01/2024	07/11/2023	13/11/2023

At Taunton and Horncastle, the four [redacted] varieties produced extremely high fresh weights compared to *Miscanthus* plants harvested at the same time. They had fresh weight yields of between 10.4 kg and 12.3kg per plant compared to *Miscanthus* x giganteus of 0.5kg and Nagara at 0.7kg at Taunton and between 4.0 and 8.6kg at Horncastle compared to *Miscanthus* x *giganteus* of 0.49kg.

Cold tolerance is routinely assessed in NEF variety trials after the first frosts in autumn. However, as it was clear that the [redacted] would not retain plant tissue over winter, and should therefore be harvested in autumn, so no cold tolerance assessments were conducted. Unfortunately, almost all the [redacted] plants at all five locations failed to survive the winter of 2023/4. They were replanted at some locations in spring 2024 but a different approach to testing them was adopted at the Taunton location. The lack of winter hardiness of [redacted] indicated that in the UK they could possibly be directed at the Anaerobic Digestion (AD) market with a late autumn harvest similar to Maize. NEF already had working experience with *Miscanthus* as a potential feedstock for AD plants dating back to 2014.

Miscanthus and [redacted] material from the 2023 harvest at Taunton was analysed at one of only two laboratories in the UK approved to test to PAS110 digestate quality specification, which measures the volume of biogas a feedstock will hypothetically generate based on the nutrient content and a series of accepted conversions. The samples are also tested for crude fibre, crude protein, crude fat (Oil A/Oil B), ash, and moisture. The 2023 results highlighted a considerably lower dry matter content and a very low gas yield from [redacted] compared to *Miscanthus* (Table 7).

Table 7: *Comparison of some AD characteristics of* [redacted] *and Miscanthus.*

Analysis	*Miscanthus*	
Crude protein %	0.9	1.1
Crude fibre %	17.2	5.0
Oven Dry matter %	37.5	13.6
Moisture %	62.5	86.4
Total Gas Yield m^3/t	206.0	65.7

In spring 2024 a trial was planted to provide analytical samples from the four [redacted] varieties for further AD testing at the end of the 2024 season (Figure 29).This trial compared the four varieties of [redacted] obtained by NEF, with a commercial Maize crop that was being grown in a neighbouring field for a local AD plant. This trial was harvested on December 2nd and the dry matter content of the four varieties ranged between 12.1% and 12.5% compared to Maize at 56.6%. The gas yields were therefore very low compared to that of maize (Table 8). The responses of the four [redacted] varieties were very consistent and the results from this trial initially suggest that they do not appear very suited for AD feedstocks. However, the wider data base that has been accumulated in other countries suggests that carefully targeted harvest dates, which demand a larger trial programme than was available here, could identify the optimum harvest date (% tissue moisture content) to maximise Gas yield and make [redacted] competitive with other crops currently used as AD feedstock.

Table 8: *AD analytical data from four* REDACTED *and Miscanthus.*

Analysis	Maize		P70	P71	P72
Crude Protein%	4.0	1.9	1.5	1.5	1.9
Crude Fibre %	6.2	3.8	4.0	4.3	4.2
Oven Dry Matter %	56.6	12.2	12.5	12.1	13.1
Moisture %	43.4	87.8	87.5	87.9	86.9
Total Gas Yield M3/t	308.0	54.4	56.4	57.0	61.0

The specimen trials have produced very clear guidance at the second harvest stage of the production cycle as to which varieties appear to be the highest yielding. Further data is presented in the replicated yield trial summary.

4.1.9 **Results:** Replicated Variety Trial

The initial delicate condition of much of the imported varietal material – and the extended timeline required before it became suitable for field planting – affected the availability of varieties for inclusion in the replicated variety trial. Notably, the issue of certain varieties exhibiting low success rates in tissue culture has also been observed in sugarcane. When varieties from different sugarcane breeding programmes were entered into tissue culture, several were found to be poorly responsive to these techniques.

A total of 23 varieties were prepared for planting in the variety trial in 2023, the three replicated plots of each variety in the variety trial requiring 72 plants in total of each variety. Unfortunately, not all the varietal material imported during 2022 and 2023 reached that target number of plants to allow them to be included in the variety trials in 2023. A further trial with the missing candidates, plus controls, has been planted in 2025. The three replicated plots in the trial each contained 24 plants, in four rows of six plants. The central eight plants in each plot were used for all agronomic and yield assessments. This format was designed to replicate, as near as possible, the plant spacing that would be used in commercial crop planting.

The varieties in trial were:

Miscanthus: 15 varieties, composed of 12 varieties in replicated plots and three varieties as single yield plots due to lower plant numbers.
Energy cane: 3 varieties.
Miscane: 3 varieties.
[redacted]***:*** 2 varieties.

The exceptionally dry June in 2023 unfortunately delayed planting until after some rainfall events in July. The planting date at Taunton in 2023 was 4th July which was 16 days later than the final specimen plot planting on 14th June at Taunton (Figure 30). No yield data was collected from the trial in spring 2024 as the plant material was small when entering the winter of 2023/4 (Figure 31). However, to provide some yield data within the Project timeline, a harvest was conducted in 2025 (Year 2). Yield data from perennial high biomass grasses is usually only presented for comparative purposes from the harvest in the third year. In November many plants were only just starting to senesce indicating why high biomass grasses are not harvested until early spring. The variety trial was harvested on 1st April 2025. The dry weight yields (Figure 34) rank M7 as the highest overall yielding *Miscanthus* in the variety trial and it was also the highest yielding in the specimen trials.

However, *Miscanthus x giganteus* is recorded as the second highest yielding in the replicated variety trial, a result which contradicts the five specimen trials yield data where it was placed 7th, 8th, 9th and 12th (twice) in ranked yield order. Extensive analysis of stem numbers/plant, stem thickness and total footprint of each variety type within a plot, has not identified any differences that relate to this recorded yield difference. Stem numbers of several varieties recorded over three seasons (Figure 35) clearing support the existing knowledge that Nagara has higher stem numbers per plant than other varieties. However, the other six varieties that have three seasons of data collected do appear to have quite similar stem numbers/plant, much lower than those of Nagara. Stem numbers would therefore not appear to be related to the higher yield performance of *Miscanthus* x giganteus. The data presented in detail from the two trialling formats is from year two harvest in both cases, so the data from the third harvest, in spring 2026 will be interesting. An image of the Taunton trial site is provided in Figure 36.

One *Miscanthus* variety has proved very frustrating to trial but does appear to have excellent potential. The first specimen trial conducted by NEF, planted in 2021 to test the specimen trial format, contained the variety M5. This variety subsequently proved to be difficult to multiply and did not appear in all the specimen trials or the replicated variety trial. However, referencing back to Figure 12 indicates that it was the second highest yielding variety after four harvests and considerable effort is being directed at multiplying this variety for more extensive testing.

[redacted]

[redacted]

4.1.10 Conclusions

The information from the five specimen plot locations gives a clear indication of the range of yield performance across 14 *Miscanthus* varieties. These yield figures, presented as dry weight in kg/plant, are from single plants, so not a conventional commercial field planting, but they do represent relative performance all in the same circumstances. The normal control variety, *Miscanthus* x giganteus, has a yield of 9.34t/ha, when presented at the conventional planting density of 15,000 plants ha^{-1}. This extrapolated yield value (Table 9) is very close to the average yield of the variety, in a commercial crop, when three years old. Six varieties are demonstrating yields of over 15 t/ha at the conventional cropping density. These have potential yields of 16.8 to 24.6t ha^{-1} (at 15,000 growing plants ha^{-1}) and of 20.1 to 29.5 t ha^{-1} (at 18,000 growing plants ha^{-1}). The performances of M3, M5 and M6 reflect the fact that these were the most difficult plants to move from tissue cultured plantlets to 'field ready' plants so their poor performances can be attributed to weaker plants when initially planted. This group of material is now showing strong growth potential and continued evaluation is warranted.

The dry weight yields from the five locations of specimen trials and the single location yield trial rank M7 as the highest overall yielding *Miscanthus*. There is also clear consistency in the specimen trials as to the varieties that feature in the top six yielding varieties. The yield results in the variety trial also indicates the performances of these top six candidate varieties agrees closely with their performances in the specimen trials. However, in the variety trial, the yield of the control, *Miscanthus* x giganteus, is a higher-than-expected result which is difficult to interpret, this was attributed to two factors. Firstly, when planting larger trial areas there were more differences in the types of starting materials that had to be used, between tissue culture plants and more developed vegetative propagules. Secondly, optimum planting densities for *Miscanthus* x giganteus have been developed based on stem populations. For new varieties optimal planting densities have not yet determined. Continued evaluation of the yield trial will allow these factors to reduce and be evaluated in more detail respectively.

The [redacted] material has been slow to establish, growth towards the end of the project, in early Spring 2025, warrants continued evaluation of this genera as a high-yielding energy crop for the UK.

Table 9: *Assessment of yield potential of the most promising energy crop types evaluated in the project, based on extrapolation of the plant weights to commercial field planting densities.*

Variety	Weight (Kg)	Yield t/ha	
		15,000/ha	18,000/ha
M7	1.64	24.55	29.46
M27	1.61	24.09	28.91
Nagara	1.51	22.66	27.19
M28	1.44	21.57	25.88
M8	1.27	19.09	22.91
M9	1.12	16.78	20.14
M15	0.81	12.22	14.66
M14	0.68	10.17	12.21
C. Illinois	0.68	10.16	12.20
M13	0.65	9.68	11.62
MxG	0.62	9.34	11.21
M5	0.59	8.92	10.70
M6	0.49	7.33	8.80
M3	0.18	2.76	3.31

4.2 WP 2 Propagation

All figures referenced in this section are provided in Annex 2.

4.2.1 Conventional Propagation Systems

This project evaluates a range of energy crop crops spanning a range of vegetatively propagated species and varieties within these species. All are sterile types propagated using vegetative plant parts of either the stem or rhizome. Most energy crops only have one sufficiently efficient route to be used commercially using stem or rhizome sections (Figure 39). Other routes are often not efficient enough (in terms of percentage survival) to be used commercially. One alternative option is to use tissue culture, which is cloning to make new plants. The energy crop options in this project were assessed to determine those most suited to be propagated from stem and rhizome cuttings. Tissue culture has been proposed as the route to commence multiplication so a new variety can be ramped up to high volumes to allow alternative propagation systems from this project to expand material further and at a lower cost. Firstly, the conventional propagation systems of sterile energy grasses were considered.

Several current commercial systems are used to scale the types of (sterile) energy crops in this project, and these are listed below.

Field Stem Propagation: Systems used extensively in the global sugarcane market and to a lesser extent (in terms of area) in the production of [redacted]. Two variants of stem propagation for these crops exist that are implemented at scale.

Billet Planting: This is where a section of the stem is planted in the ground (Figure 40). This system exists in a fully automatic system where billets are cut into smaller pieces (30cm) and planted automatically or a system with some field labour where long sections are laid in the ground (> 1m long). The penalty for using a more automatic system is a lower multiplication factor. The lower multiplication with this system is because the billets are cut into shorter sections, which causes more damage to the nodes, and they are also mechanically not hand planted, so higher numbers of billets are planted per hectare (ha) of land in the less precise mechanical option (which also reduces the multiplication factor).

Meoisi Planting: The second stem propagation system is called the Meoisi system, where in year one, 10% of a field is planted with Sugarcane (as propagation material), the other 90% with soy, and after 10-12 months, the soy has been harvested. The whole field is planted with the stems from 10% of the land.

Conventional Rhizome Propagation: Systems used in *Miscanthus* growing to field propagate *Miscanthus*, where rhizomes are multiplied in the ground, extracted from the soil before being broken up and graded, and then replanted (Figure 41). Rhizomes are below-ground stems, so there are a lot of parallels between this system and billet planting in Sugarcane, but they have a lower level of commercial use in terms of area annually.

4.2.2 New Propagation Systems

New Energy Farms (NEF) developed and patented[3] the CEEDS™ technology, which is an artificial seed system for crops that do not produce conventional seed. It was developed to scale and establish large-scale crop production areas for sugarcane crops. With a common manufacturing system that can be adapted for grasses propagated from either stem or rhizome material. CEEDS™ are small, uniform, coated units that are directly drilled into the field. They are produced horticulturally in CEEDS™ bio-factories, which are horticultural units with a much smaller footprint than conventional field propagation (Figure 42) and can produce 500 to 5,000 ha of planting material per year. The most developed end market for CEEDS™ is Sugarcane, which was licenced in Brazil. CEEDS™ provides multiple benefits. These include a significant reduction in planting weight and faster, safer planting. Plus, disease-free material, faster variety introduction, and field delivery for crop protection and biologicals are included in CEEDS™.

For propagation of energy crops that favour stem propagation, the NEF CEEDS™ system is a horticultural system developed for Sugarcane and has three key stages, starting with tissue culture tissue, which ensures small, disease-free material. Secondly, the tissue culture plants are grown under specific conditions [redacted]. This is then processed automatically and treated before being used in the final stage. This stage can be repeated, which reduces the amount of starter tissue culture plants required but increases the duration of full propagation. The final stage is the hardening of the nodal material in a high-density horticultural system under specific stresses. This material is

[3] [redacted]

then used as the final propagule, treated with crop protection and nutrients, and then coated in a biodegradable polymer to protect it during storage and transport. These uniform planting propagules, about twice the size of a wine cork (Figure 42), are then planted using fully automatic planters based on modified potato planters. These planters do not need any manual labour, and the material is planted in the ground without the need for irrigation. As production is horticultural, there is more flexibility when crops are grown and scheduled for a specific planting window. For energy crops that propagate via rhizomes (such as *Miscanthus*), not stems, the CEEDS™ system had to be adapted. The start of the process (via tissue culture) will be the same. The aim is to produce approximately 40 times more rhizomes per ha of space than conventional field material. However, the key benefit will be a significant reduction in space, and being horticultural, it will have a high operational efficiency. Field rhizome production, in contrast to stem harvest, is much more weather dependent and often operates on only one every three days, being suitable to lift rhizomes in terms of the weather. This risk can be mitigated by working on land types (lighter sandier soils) and regions where field access is improved with less operating time lost due to weather. This makes rhizome production suitable for scaling to a point, but there is a limit to how much capacity a fixed machinery investment can process inside a three-month harvesting window. As with stem propagation, rhizome CEEDS™ has some flexibility for timing, so planting could be scheduled for the autumn or spring, or over both planting windows of six months. The purpose of this project is to develop these systems for *Miscanthus* and other rhizome-propagating energy crops.

4.2.3 Nodal Production in Energy Crops

For current *Miscanthus* rhizome production in the field, 0.4 million cuttings are produced per ha, with an average weight of 40g. This project at the start targeted [redacted] (as stems grow vertically, more nodes can be produced per unit ground area). For Sugarcane, NEF has shown that the current field rates of production of one million nodes per ha can be increased [redacted].

Development work screened all current species and varieties to determine if stem or rhizome propagation was the optimum route for their propagation (Figure 43). *Miscanthus* was the only species suitable for rhizome production from all variants tested. Academic studies have previously evaluated *Miscanthus* x giganteus stem propagation[4,5], (the main variety grown commercially in the UK). These studies found a high density of nodes per ha, but the percentage of germination under cooler field conditions was insufficient for commercial use. Based on this, *Miscanthus* x giganteus is known to be a recalcitrant stem propagator, which was reflected in the results. Assessments showed that *Miscanthus* x giganteus had very low stem propagation potential compared to a variety with known stem propagation potential, *Arundo donax*.

[4] *The influence of propagation method and stand age on Miscanthus x giganteus performance in Iowa, USA. PhD Thesis Nicholas Boersma, Iowa State University. 2013.*

[5] *Establishing Miscanthus x giganteus crops in Ireland through nodal propagation by harvesting stems in autumn and sowing them immediately into a field. Biomass and Bioenergy. 107 345-352. O'Loughlin et. al 2017.*

However, when examining greater diversity in *Miscanthus* material, out of ten new *Miscanthus* accessions, three have some potential for stem propagation.

Trials were completed on nodal production of mini rhizomes with different horticultural growing containers, mediums and planting densities using biotic stressing treatments. Overall, the results (Figure 44) show that a potential of up to [redacted]. This confirms the target yield and size of propagation material can be produced using CEEDS™ intensive node protocols for *Miscanthus*. A small target size of material is required to support using small cell horticultural trays for a high production volume of material in the hardening phase. In this work, Anaerobic Digestion (AD) digestate was shown to have the same potential as commercial compost. This was further enhanced by using specific fertigation regimes to increase the yield and quality of the material. The most promising development work identified systems for hydroponic rhizome production. The material produced was of high quality and had an easier potential for processing, with the reduced or zero soil content in the system.

Trials were also completed on nodal production of mini stem material, using a tray-based horticultural system to maximise the production per m^{-2} of growing space (Figure 45). The results (Figure 46) show that all varieties tested can meet the minimum target of [redacted]. This confirms the target yield and size of propagation material can be produced using CEEDS™ intensive node protocols for energy cane types. A small target size of material is required to support using small cell horticultural trays for a high production volume of material in the hardening phase.

4.2.4 Hardening

The hardening phase is used to make CEEDS™ propagules with multiple buds that are in a dormant form so they can be coated and drilled like a conventional seed. Develop work focused on validating production protocols in the key areas of material planting, substrates, and treatments to enhance germination and production stress treatments. Firstly, the mini nodal material produced for the hardening phase has high germination viability.

The material was shown to have a high number of buds per kg, which allows a smaller weight to be planted to deliver the same number of growing buds (points) per ha in the field. [redacted]. The production of mini nodal materials (rhizomes) can be completed under a range of temperature conditions, including the UK. For mini nodal material (stem), the production potential in the UK was limited due to the lower temperatures, which was reflected in some of the field yield performance (Section 4.1 main report, further details in Annex 2). Work in this project showed that energy cane types can generate a large number of nodes per ha of growing space, with a high germination efficiency of over 89% (Figure 47) using single pieces. Two approaches were followed to ensure a high percentage of germination. [redacted].

Secondly, to enhance germination, a range of treatments were developed to maximise the percentage of [redacted]. These were in four growing mediums, hormones, nutrient soaks and carbohydrate soaks. This is important as it is a low-cost means of enhancing germination to reach the commercial standard of 95%. It is also a means of developing custom treatments for varieties that are more recalcitrant to propagation. Some varieties (Figure 47) show low germination, below the commercial threshold.

For CEEDS™ these treatments of material prior to planting allow the process to be cost-effectively modified for varieties that are in demand, which is not possible for field production. The work has also demonstrated that small pieces of vegetative tissue (as long as they have sufficient nodal pieces on them) can generate viable and vigorous planting material and do this with a high level of horticultural space efficiency. The tray size used for hardening ranges from an internal volume of [redacted] of growing space. This is an important component of the production model as the hardening phase uses up the most space as part of the CEEDS™ bio-factory (Section Five main report, with further details in Annex 5), so a density above [redacted] of growing space is required.

Growing Mediums

In terms of growing mediums, a range of substrates were tested to determine if lower-cost and non-peat-based mediums can be used instead of conventional commercial growing mediums using peat compost as a control. Using a pot-based screening trial (Figure 48), Anaerobic Digestion (AD) digestate was found to be the best alternative to conventional peat substrate, showing 100% germination compared to peat as a control (Figure 49). Other mediums tested had down to 20% of the germination of the control. This germination testing of mediums was completed in both pots (Figure 48) and in trays. In addition to germination, the mediums also need to support strong root development, as the roots are also used during the hardening phase to ensure the final propagule is strong and does not fall apart during coating, transport and planting. The root development using untreated mini nodal material was also evaluated using different growing mediums. Again, AD digestate was found to be the best alternative to conventional peat, showing that 100% of the root development scores were achieved with conventional peat as a control. Some mediums that had low germination directly correlated to low root development. There was a focus on evaluating other low-cost options that may potentially reduce the cost of production and the use of waste materials. Digestate was the only successful medium, such as using just coffee grounds, or proportions were generally negative on root development compared to control. These treatments were used to develop commercial protocols for the hardening phase to produce tray material with high germination and vigour. Growing structures for both the intensive node and hardening phase must be low-technology outdoor growing systems, using low-cost benching or pallets with trays and overhead irrigation.

Carbohydrate, Nutrient and Priming Treatments

To increase the germination percentage and improve the vigour of mini-nodal material, a range of treatments were assessed, including nutrient soaks, priming, and hormone applications. Full-spectrum nutrient solutions were used for soaking, alongside specific nutrients known to be effective on related genera. Overall, these chemical priming treatments required longer uptake periods than non-chemical methods, typically ranging from 1 to 24 hours. It was observed that water alone – or non-beneficial nutrient solutions – for 24 hours could reduce germination compared to a 1-hour water soak (used as a control). This reduction is likely due to the leaching of beneficial nutrients, hormones, and carbohydrates from the vegetative material during extended treatment. As such, the treatment solution must provide a net benefit to the plant material. Examples are provided in this report illustrating how these treatments were developed to enhance germination.

Carbohydrate soaks are used in tissue culture to encourage growth on shooting and rooting mediums. Work was undertaken using both nodal materials from *Miscanthus* and energy cane. Firstly, for nodal material, there is often [redacted]. A range of carbohydrate solutions were also evaluated in screening trials to evaluate the best level, higher concentrations were found to be optimum, In *Miscanthus* (Figure 50) the carbohydrate soak resulted in faster earlier germination. When tested on energy cane, the same response was found overall, earlier germination was achieved, but over the full assessment time, water alone was better. Overall, this priming has shown that the combined treatment solutions will need carbohydrates for earlier germination, but layering with the nutrient additions will also be required to provide both earlier germination (which enhances overall growth) and greater germination for production efficiency.

Using a nutrient solution priming on *Miscanthus*, a 1-hour water soak was the optimum treatment, compared to lower germination (than control) with all 24-hour treatments, lower than the same with a 1-hour treatment (Figure 51a). Final plant weights were also reduced in line with the germination efficiencies. Treatments were adjusted to focus on specific nutrients with proven efficacy in other vegetative crops for 24 hours. Firstly, water soaking alone does provide some benefits, but priming with water containing phosphate was significantly better than water alone. However, the biggest increase in germination was found with specific nitrogen formulation treatments. Here, 87% of buds on the rhizomes germinated, compared to 47% with no water soak and 64% with a water soak alone. Shoot height after 42 days of growth was also double with the nitrogen formulation soak, compared to water and no water soak treatments (Figure 51b).

The final aspect of layering the treatments is the addition of hormones. Treatments were developed for a range of hormones, which were found to significantly increase germination compared to control. The best rates and hormones were capable of doubling the germination compared to controls (Figure 52).

These combined treatments have been developed into treatment protocols, which are part of the wider set of protocols described in the commercial section (Section 5) to be used as part of forward commercial licensing.

4.2.5 Coating

The storage and germination of CEEDS™ propagules require a coating and treatment for optimal performance. This will reduce water loss during storage and early planting, plus prevent mechanical damage at planting. The coating was also developed to contain crop protection products applied to reduce bacterial and fungal infections during storage and in the soil during early growth. Approximately 30 formulations were tested for coating materials to identify those that were physically effective, could be applied easily, were not phytotoxic, and were low-cost materials. Images of material being coated for testing are shown below (Figure 53). The best material identified was a biodegradable polymer which is a very effective non-phytotoxic material, and it is low cost and bio-degradable in the soil. Previously, wax was used, but in some instances, it was found to be phytotoxic to early root development and growth (Figure 54).

4.2.6 Field Delivery

Field delivery work focussed on three areas: of CEEDS™ coating prior to planting, developing a fully automatic field planting system, and finally, development of protocols for field planting and testing performance.

Fully Automatic Field Planting

The current planting of sterile, high-yielding perennial energy grasses is based either on rhizome systems with a focus on *Miscanthus* or stem-based systems with a focus on Sugarcane. Both are effective and have been commercially scaled to different extents. Sugarcane stem propagation is used to replant over five million hectares (ha) annually (out of a global crop area of circa 25 million hectares) in the global sugarcane market, which has developed over the last 500 years. The much younger *Miscanthus* market has developed rhizome systems, which have been the main establishment system for the estimated 30,000 ha planted globally to date (mostly in the EU and North America). While both are effective, there still remains a step change in the difference between how these crops are planted and the much larger area of crops drilled via seed. For example, Sugarcane uses 15t – 20t ha^{-1} of stem material to establish one ha of crop. A field of UK wheat would use around 200kg of seed, which is 75 times lower. UK *Miscanthus* is circa one tonne (based on rhizomes) and is still five times higher. This places a challenge in terms of logistics, speed and field efficiency of planting, as well as physically moving and planting a much higher volume of plant material. There are also additional challenges in that planters for larger volumes of vegetative material are harder or impossible to integrate with modern technologies such as minimum tillage.

The CEEDS™ propagules are similar to seed potatoes in size, and existing potato equipment was found to be suitable machinery to utilise. Working with an established potato planting manufacturer (Standen Engineering), NEF initially tested CEEDS™ material inside potato planting equipment for suitability (Figure 54). The internal separation and collection mechanism from a potato planter was bought and tested to ensure it can accurately pick up CEEDS™. This testing showed that this system could work very effectively. Following this, multiple Computer Aided Design (CAD) drawings were developed to arrive at a final working system for planting energy crops in the UK (Figure 56). Subcontractors with long-term experience planting energy crops in the UK (WH Loxton Ltd, and *Miscanthus* Nurseries Limited) were used to develop the design. Following refinement of this CAD work, the final planter was manufactured (Figure 57).

CEEDS™ material was tested under both field and greenhouse conditions. Material was field planted in Spring 2022 (Figure 58 & Figure 60). This demonstrated the vigorous growth of the material, showing the early germination and the final plots at peak growth in August (Figure 59).

The CEEDS™ production process delivers a step change in reducing the space requirement for the propagation of perennial grass and enhancing the logistics of production. For space, a CEEDS™ bio-factory for 1,000 ha of material (20 million units) requires just under 6ha of production space. This is already significantly lower than field rhizome production for *Miscanthus*, which is nine times larger at 55 ha for the same output (Figure 61). Development work identified that a modified v2 [redacted]. Combined, these improvements would reduce the production time by 50% and the operating footprint from just under 6ha to 1.7, a 67% reduction (Figure 61). In addition, this leads to significant cost savings and allows production sites to supply a wider region with material.

4.2.7 Paludiculture CEEDS™

Work on paludiculture in the project has focussed on developing solutions for *Typha* based on the stakeholder focus and demand for this plant. The current commercial supply of this product in the UK market is restricted to low volumes of high-cost plug plants produced via manual propagation. In addition, the stakeholder demands for planting *Typha* also require products that can be delivered onto wetland planting by different planting means, including via drone, for which plug plants are unsuitable. As a result, three propagation routes for *Typha* have been developed for this project's field delivery component. The work was focused on three areas, all of which developed commercial supply products. Balls - For drone or other fully automated planting (Figure 63, images A and D). Work has also successfully tested these balls and confirmed that they can technically be planted and distributed using a drone.

Plug Plants: Systems were developed to allow cost-effective automatic planting and production of plug plants in trays for manual or semi-manual planting. Equipment has been designed to produce this system, which, for the first time, delivers a system for large-scale automated production of *Typha* plug plants (Figure 63, images B and E).

CEEDS™: Based on the two products above expanded protocols were produced to convert this material into CEEDS™ which can be coated and automatically planted (Figure 62, images C and F). This produces a product that, for the first time has the potential of both automatic production of a *Typha* plant propagule and automatic planting. The CEEDS™ can be planted using the same type of planter developed for field planting (Figure 62, image F) but with modifications to operate on different (flooded) ground conditions, like the equipment used to harvest *Typha* biomass.

4.2.8 Priming

Priming plant material to deliver faster and higher levels of plant emergence and establishment is a technique first developed and reported by Professor Walter Heydecker in 1973[6]. It is now a well-established technology applied to the seeds and bulbs of many plants. More recently, the term priming has also been used to describe a response in some plants that can "prime"

[6] *Heydecker, W., Higgins, J., & Gulliver, R. L. (1973). Accelerated germination by osmotic seed treatment. Nature, 246(5427), 42-44.*

appropriate defences and deploy faster, stronger responses to infections and even grazing animals. However, this is not the target of these studies. The work specifically addressed the potential to prime rhizomes of *Miscanthus* to maximise bud emergence and stem numbers in commercially planted rhizome crops.

Miscanthus rhizomes are not only very unusual shapes but are bulky and, when planted at 17,000 rhizomes per hectare, can involve over 1,000kg/ha of plant material. Commercially priming *Miscanthus* rhizomes, therefore, faces two significant challenges. The production process must accommodate both the weight, and the volume of material required to plant thousands of hectares of material each year.

Priming trials in this project have been conducted every spring, spring being the conventional time to lift *Miscanthus* rhizomes. The initial experimental approaches involved soaking processed rhizomes in controlled conditions to explore the effects of:

- Different temperature treatments in the priming process.
- Different durations of treatments in the priming process.

Immediate planting after priming demonstrated slightly higher emergence levels compared to unprimed controls. When a storage treatment was added after priming (Figure 63), a seven-day storage period following priming resulted in the highest emergence levels, outperforming other storage durations including no storage. Bud emergence levels began to decline after fourteen days of storage, with the lowest levels observed at twenty-one days of storage. The priming response does appear to be very short-lived in the rhizome.

The converse trial was also conducted, aiming to evaluate the effects of storing the rhizomes first and then applying priming treatments (Figure 64). The bud emergence results were almost the opposite of those recorded when the priming treatments were applied before storage. The treatment with the lowest bud emergence was storing rhizomes for seven days, priming, and then planting. Storing for 14 or 21 days, then primed and planted, brought the % bud emergence back to about the bud emergence level of the freshly primed rhizomes.

The project also explored the response of rhizomes to priming treatments when lifted earlier than the conventional timing (Figure 65). The conventional time to lift *Miscanthus* rhizomes for processing so they can be used to establish new commercial crops is from March through April each year. An exploratory trial to investigate earlier lifting of rhizomes was started by testing rhizomes from November and January. The rhizomes were then planted in propagators either directly or with priming treatments.

Rhizomes showed a very clear response to the date of lifting. The percentage of bud emergence increased steadily from November to March. This work was repeated with different sources of rhizomes, and the optimum priming treatments did differ between the two locations. During the project, a greatly increased number of samples of rhizomes were primed from commercially lifted crops after they had been processed and stored in storage before planting. Unfortunately, the results do not convincingly point to any specific priming treatment as optimum. Whilst disappointing, it does suggest that the diverse nature of the origin of each rhizome batch for processing into material for new commercial plantings presents material with a wide range of physiological characteristics that do not respond uniformly to priming. The reliability of that response, the scale of investment required to provide a priming capability on a processing line, and the expected introduction of new planting technologies have led to the conclusion that investment in priming could not be justified, but the system could be applied to other propagation systems such as CEEDS™ where there is more uniformity of the material being treated.

4.3 WP 3 Biologicals

4.3.1 Introduction

Capturing synergies that exist between crops and endophytes (beneficial fungi and bacteria) is a rapidly developing technical area in the world of agriculture. The market for Rhizobium (soil bacteria that can fix nitrogen) and Mycorrhiza (fungi that can form symbiotic associations with the roots of a vascular plant) is already considered to be about $1 billion worldwide. Members of the Family *Poaceae* (Grasses) are responsive to microbials, a generic term favoured to describe these organisms. In New Zealand, endophytes are now commonly used in planting new grass pastures where, once associated with the growing grass plants, they can reduce insect attacks. The relationship that they can establish with the plant varies and ranges from symbiotic to pathogenic.

The benefits of microbials are documented to include growth promotion, nitrogen fixation, disease control, insect control, stress (drought management) and tolerance of heavy metals. However, microbial associations can be very plant-specific and climate specific.

Initially, NEF explored the possibility of importing into the UK-specific microbes with known benefits of associating with *Poaceae,* as seen in New Zealand, to test under UK conditions, but this was not possible under current importation regulations.
The studies, therefore, focussed on four research areas:

- Understand the microbial populations that currently exist in soils and on the root systems of commercial *Miscanthus* crops in the UK.
- Evaluate the extent to which associations can be developed between *Miscanthus* and the wide range of microbes that are already available commercially in the UK market.
- Assess any longer-term effects of microbial associations in a controlled field trial situation.
- Explore the integration of microbials into CEEDS™ propagules.

4.3.2 *Miscanthus* Microbial Field Sampling

NEF's collaborator in this work package was the University of Warwick**.** In the first phase of the collaboration, NEF undertook what is believed to be the largest microbial sampling programme ever undertaken in the UK on *Miscanthus*. The sampling of commercial *Miscanthus* plantings took place over a period of three weeks in October 2022 in crops from South Cornwall into Wiltshire. Soil and rhizome root hair samples were obtained from 22 crops with initial planting dates from 1998 to 2020 and delivered in chilled storage to Warwick University for microbial association analysis.

DNA extracted from the 110 root samples revealed very high numbers of both fungi and bacteria. The main findings from the University of Warwick's report relating to fungal and bacterial presence in this comprehensive survey are listed below:

- Significant differences are present in fungal diversity between locations (Figure 66).
- Across sites and age categories arbuscular mycorrhizal fungi and pathogenic fungi were present in very low abundance within the roots.
- *Trichoderma spp*. which are often used in industry as plant growth-promoting inoculants, were not detected in the samples.
- The dominant fungi (detected at most sites) were assigned to the *Heliotales* family. This is apparently a largely understudied fungal order, but one which is emerging as being involved in fungus-mediated nutrient acquisition by plants.
- *Periconia macrospinosa,* which is a dark septate endophyte, was abundant at all sampling locations, and this fungus is known to form mutualistic associations.
- [redacted].

The analysis of the bacterial community indicated that the types of bacteria in each of the 22 communities across the sites and *Miscanthus* age classes were actually very similar (Figure 66). The main findings are listed below.

- The high relative abundance of pathways involved in the degradation of plant biomass is linked to saprotrophic functions of the bacterial root microbiome.
- The top 20 most abundant bacterial Amplicon Sequence Variants (ASV) across sites were ones which use compounds such as methane as substrates. This high abundance of anaerobic fermentation functions points to an oxygen-depleted environment in the root zone of *Miscanthus*.
- Functional analysis indicated that N_2 fixation was not operating in the roots of *Miscanthus* at any of the sites.
- Analysis of nitrogen cycle functions showed that aerobic and anaerobic denitrification functions were present, suggesting the presence of NH_4^+ denitrification pathways.

In summary, the 22 *Miscanthus* sites had very different fungal populations, but the bacterial populations were very similar, which is an important contrast and a result that was not expected. The Functional analysis indicated that the *Miscanthus* root microbiome could be operating under an anaerobic environment, with evidence for methanogenesis pathways in which plant material is broken down. It was commented by the subcontractor (Warwick University) that *'the presence at most sample sites, of root infecting fungi known to damage some grasses, is interesting and should be further explored'*.

The full report on this work is enclosed in Annex 3.

4.3.3 Greenhouse and Field Trials with Commercial Microbial Formulations

Seven commercially available microbial formulations were obtained from UK and European companies, one of which was able to supply an endophyte which is reported from studies in New Zealand as having clear associations with *Miscanthus*. These microbial formulations were used in two trials greenhouse trial and a field trial. The greenhouse trial, using small *Miscanthus* plants, which lasted 465 days, began with a six week 'association', the time generally recognised for any root associations to begin in the soil after the microbes have been mixed in the soil before any measurements were taken. Small differences in plant height, stem number and leaf number were recorded in the *Miscanthus* plants, but no significant responses were observed to any individual treatment. Rather than harvest the trial (in view of the fact that there appeared to be no treatment differences generated) the individual plants were moved to a field trial. This was in soils of two fertility levels and lasted a further 229 days. The yields in the higher fertility area were considerably higher than in the low fertility area, as would be expected. Two microbial treatments, both with *Trichoderma*, produced the highest yield responses compared to the other five microbial treatments, but the control treatment was also performed well. The overall response from this single trial was inconclusive.

Several microbial treatments were used in the contaminated soil trials produced some interesting positive plant survival data on plants grown in heavily contaminated soils.

The CEEDS™ propagule method of planting provides the ideal to process in which to create microbial associations. Liquid or powder formulations of microbes can be applied at any time during the growing cycle of the young plants before they are coated and made ready for planting so plant material can effectively be 'associated' before it is commercially released.

The full report on this work is enclosed in Annex 3.

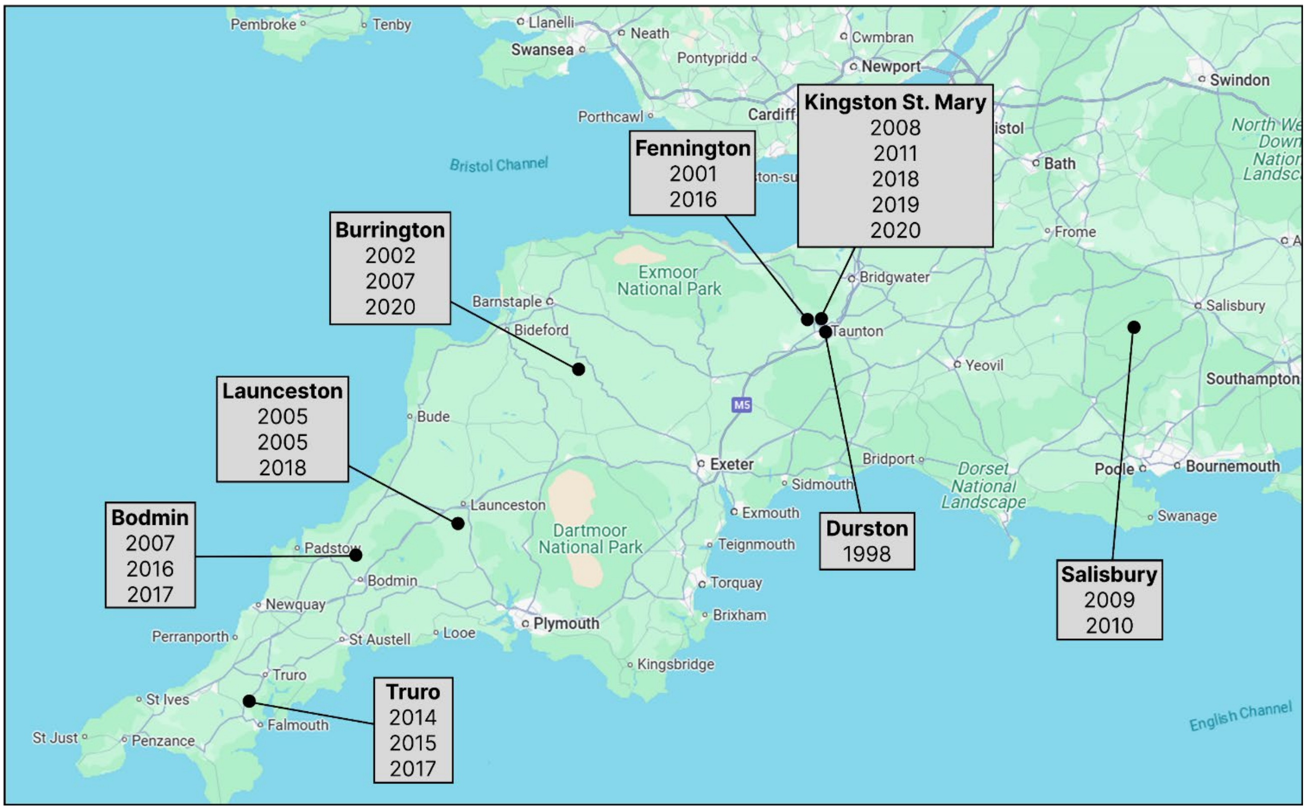

Figure 3: *Commercial Miscanthus crop locations sampled in 2022 for soil microbial associations with crops between 2 and 24 years old (at the time of sampling).*

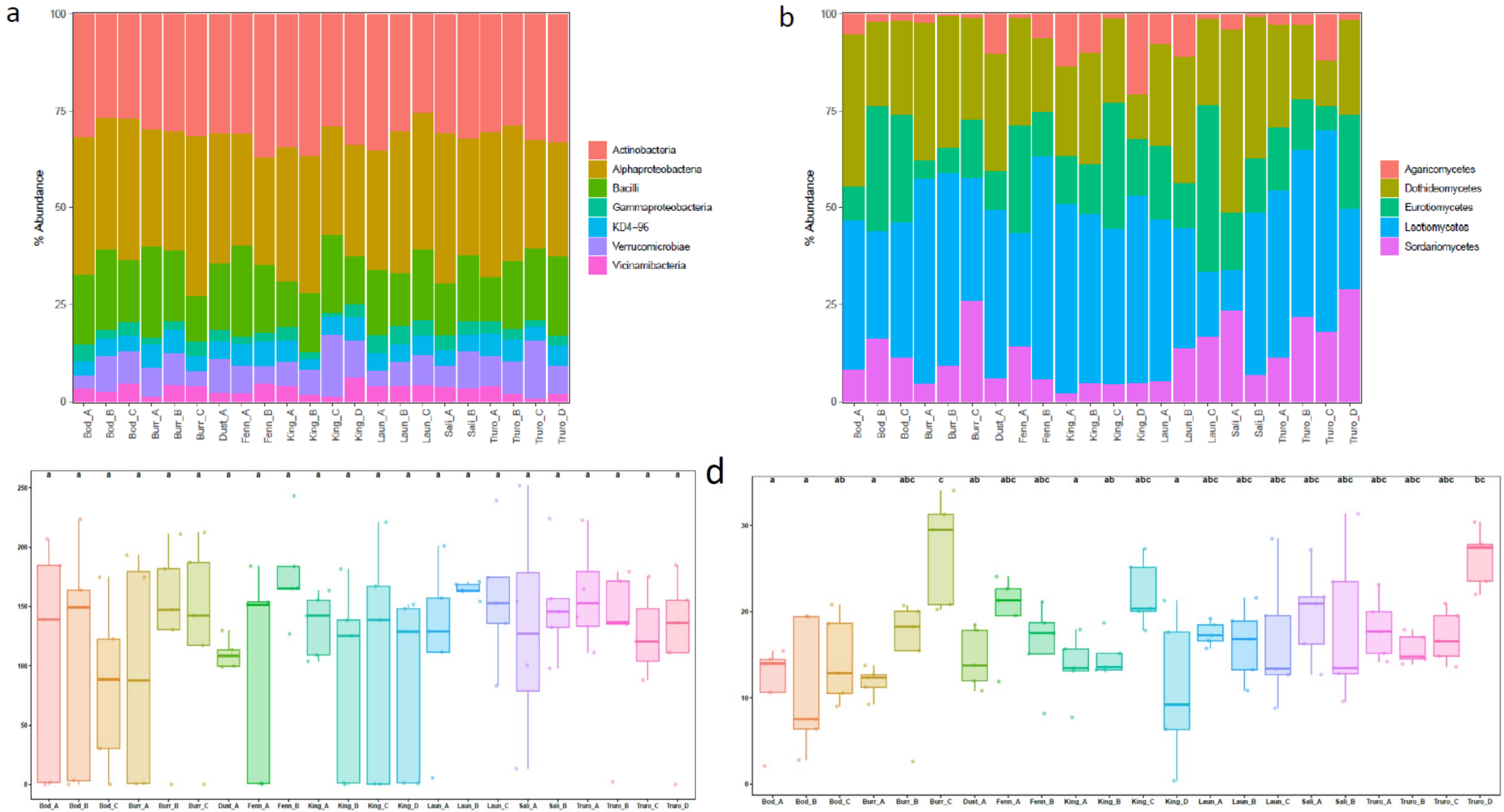

Figure 4: *Miscanthus root microbiome diversity and composition across sampling locations: (A) Relative abundance of bacterial classes; (B) Relative abundance of fungal classes; (C) Bacterial diversity; (D) Fungal diversity. Different letters indicate significant differences (P<0.05).*

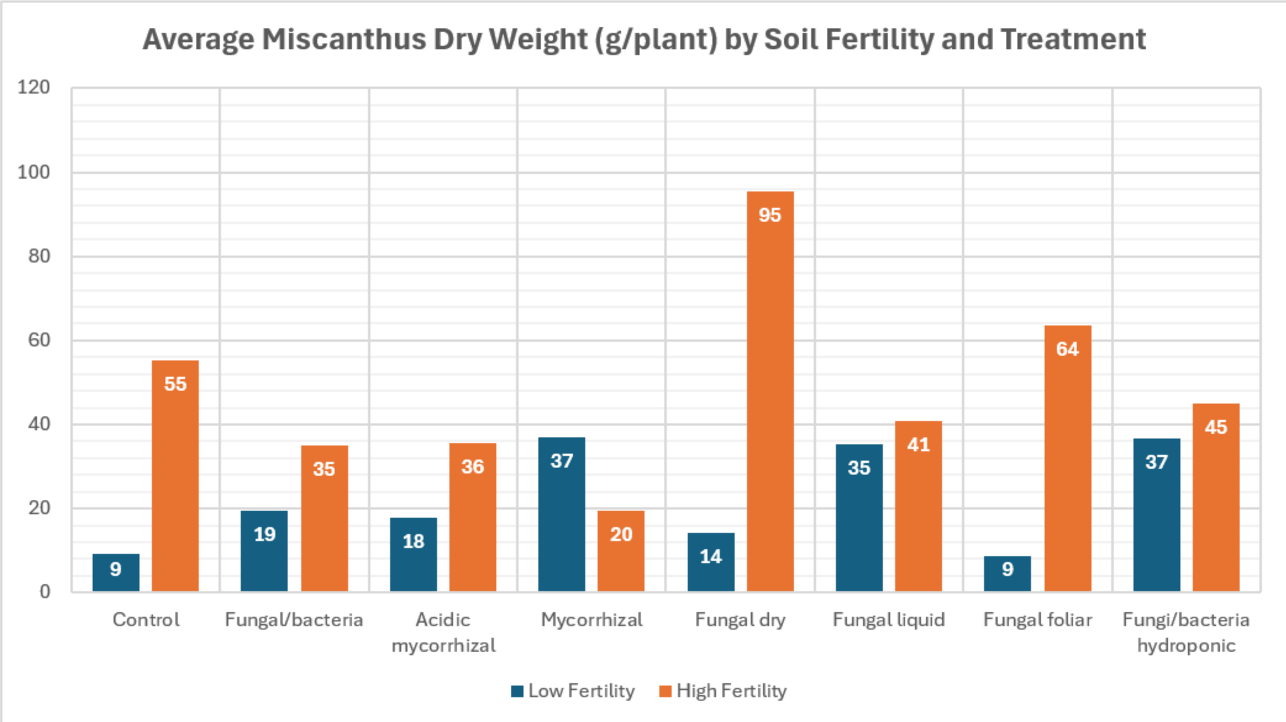

Figure 5: *Yields (dry weights) after growing in the low- and high-fertility soils of Miscanthus plants (cv. Illinois) compared to Untreated Control with seven different microbial treatments applied.*

4.4 WP 4 Paludiculture and Contaminated Land

All tables and figures for this section are provided in Annex 4.

4.4.1 Paludiculture Introduction

The urgent need to address CO_2 emissions from wetland areas has created both challenges and opportunities. Establishing new plants in wetland areas has long been difficult, but it also presents opportunities for new technologies. The UK government plans to re-wet large areas of peat soil. There are approximately 325,000 ha of lowland peat soils in the UK, which hold over 50% of the country's terrestrial stored carbon, yet less than 1% of this remains in a natural state[7]. Around 74% of these soils are currently used for farming, and the UK government plans to potentially rewet around 280,000 ha[8], with the aim of restoring them using wetland plants such as reeds and grasses – a practice known as paludiculture[9].

Globally, peatlands cover 0.4% of land area in the world and contribute 5% of total global anthropogenic GHG emissions. Worldwide, inland wetlands are critical carbon reservoirs, storing 30% of global Soil Organic Carbon (SOC) within 6% of the land surface. This clearly illustrates the disproportionate importance of wetlands. Two examples of wetlands in the UK

[7] DEFRA UK 2023. https://www.gov.uk/government/news/new-investment-in-peat-in-fight-against-climate-change

[8] https://www.wwt.org.uk/uploads/documents/2024-10-17/100000-hectares-of-uk-wetlands-exploring-the-potential-wwt-1710.pdf

[9] https://lowlandpeat.ceh.ac.uk/sites/default/files/2022-07/Defra-LP2-paludiculture-report-April-2020.pdf

are The Broads National Park, which stores an estimated 12 to 14 million tonnes of carbon and the Somerset Levels, where the estimated storage is 11 million tonnes of carbon. Wetlands, particularly areas with peat soils, such as the Broads and the Levels, are some of the UK's most valuable habitats, vital in supporting unique plants and rare wildlife. Unfortunately, over many years, some of these wetlands have been degraded by drainage, which releases carbon. In the UK, it has been estimated that an extra 14 million tonnes of CO_2e per year, nearly a third of UK agricultural emissions, could be locked up if these important habitats for nature were restored.

To replant and restore this land, a range of paludiculture crops are required. The project had discussions with groups including Natural England and The Broads Authority, which created a target list of genera of interest for wetland remediation covering *Miscanthus, Typha, Phragmites, Phalaris and Molinia*. NEF obtained the material of each genus and commenced initial studies on applying both propagation technologies on the plant material. Further meetings with remediation and paludiculture researchers, stakeholders and advisors presented clear views on why the target number of genera should be reduced, primarily based on possible end-use opportunities for the biomass produced from the wetland environments. As a result of this consultation, the primary candidate genera for initial work was *Typha latifolia* (commonly called Bulrush), *Phragmites australis* (Common Reed) and *Phalaris arundinacea* (Reed Canary grass).

4.4.2 Initial Experimentation

Current propagation methods for these 'target' paludiculture crops are based upon conventional horticultural techniques, usually of cuttings which, after a period of growth develop into plug plants. These are identical to those used for large-scale field planting of vegetable crops and are the type of plant plugs that gardeners would purchase from a garden centre. This can result in high unit costs for each plant, as shown in Section 5. Plants of *Typha* and *Phragmites* were obtained from aquatic plant suppliers who would normally supply material in bulk for use in ponds and lakes.

Phragmites and *Phalaris* have very similar anatomical structures, being rhizomatous with numerous nodes and both being in the Family *Poaceae*. The experimentation, therefore, used *Phragmites* to represent both genera. Initial studies focussed on node production in *Phragmites*, using technologies applicable to NEF's CEEDS™ technologies to try and reduce the unit cost of production. *Typha,* which is in the Family *Typhaceae,* whilst being rhizomatous, does not have nodes on the rhizomes, its vegetative reproduction occurring by the production of lateral rhizomes from the meristem at the base of the leaves. It was clear from the outset of the experimentation that this morphology would be less responsive to the current propagation technologies. Later in the project in 2023, it became clear, with more feedback from the paludiculture sector, that *Typha* had become their primary target crop, primarily because of its end-uses opportunities. However, as there was still support from the paludiculture sector for *Phragmites* and *Phalaris,* NEF retained these two genera in the trial plans.

4.4.3 *Typha*-Focused Work

Work on paludiculture in the project moved from field studies on several genera focusing on developing solutions for *Typha*, based on the stakeholder focus and demand for this plant. Earlier in this project, NEF identified that vegetative propagation of *Typha* by rhizome division was hard because of the extremely low multiplication rate and recalcitrance to fragment regeneration. As a result, further studies focussed on using *Typha* seeds as the starting point for propagation. *Typha* seed has a low germination percentage and requires pre-treatment to gain commercially acceptable levels of germination. This treatment often requires hydration of the seed, which as a result, renders most commercial systems to plant in the field or horticulturally redundant as the seed will not flow smoothly through machinery. In addition, the stakeholder demands for planting *Typha* require products that can be delivered onto wetland planting schemes by different planting means, such as drones, for which conventional plug plants are not suitable. As a result, for the field delivery component of this project, three propagation routes for *Typha* have been explored, and each has resulted in potential commercial systems to supply planting products.

The starting point for all these three planting products depended upon developing a production system that maximised the germination of *Typha* seeds. It has been estimated that each *Typha* flower spike can produce over 200,000 seeds each year which, suggests that there is no shortage of seeds. However, each seed has many hairs at its base, useful for wind and water dispersal. but very inconvenient when trying to separate and process batches of seeds. The experiments, which involved different hydration regimes and different temperatures to break dormancy and optimise subsequent growth and different light wavelengths, finally developed a combination of treatments which triggered satisfactory levels of seed germination. The 77 cell trays (Figure 86) illustrate the differences in *Typha* seed germination resulting from the different hydration, temperature and light treatments. The dramatic effect of the combination of the correct dormancy-breaking treatments is shown in Figure 87.

The successful development of treatment combinations to maximise seed germination allowed three potential planting approaches to be pursued. All three potential delivery systems are based on a specially formulated system containing *Typha* seeds, which have been subjected to variations of heat, light, hormone and hydration treatments, reflecting the light frequencies and water conditions they would encounter in the wild. The three options were:

Balls: This presentation of the germinating seeds is based on creating a spherical planting propagule, the seeds being contained within a hardened casing (Figure 88A). By producing robust balls, drones or other fully automated planting systems suited to wetland situations, can satisfactorily distribute the propagules onto the soil or water surface (Figure 89D). Prior work has already demonstrated that these can technically be planted/distributed using a drone.

Plug Plants: Using the same initial system for the ball, systems were then developed to allow cost-effective automatic planting and production of plug plants in trays for manual or semi-manual planting. This delivers two benefits, firstly the optimal treatments deliver full, vigorous tray germination (Figure 88B) secondly, it solves the issue of being unable to commercially automate wet seed planting and for the first time, delivers a system for large-scale automated production of *Typha* plug plants containing germinated seeds. This type of material could also be hand-planted in the field, (Figure 89E).

CEEDS™: Based on the starting position of the plug plants, expanded protocols have been produced to convert this material into CEEDS™ that can be coated and automatically planted. Earlier, it was noted that using *Typha* rhizome material was not effective. However, the rhizomes of this plant are very robust, and a protocol has been developed to use the resulting material from the other production protocols to develop a dormant CEEDS™ product [redacted]. This produces a product that, for the first time, has the potential for both an automated production process for a *Typha* plant propagule and automatic planting. The CEEDS™ can be planted using the same type of planter developed for field planting (Figure 89F) but with modifications to operate on different (flooded) ground conditions, like the equipment used to harvest *Typha* biomass.

4.4.4 Contaminated Land Introduction

Over two million hectares of land in the UK and Europe is contaminated, and in the 2% of the UK land area that is classed as contaminated 60% of it is metal contamination. The spoil heaps from over 1,600 coal mines, mainly in the north of England, Scotland and Wales, covered 22,000 hectares of land in 1966, but successful reclamation work, primarily by the Coal Authority, has reduced it to 2,000ha. However, it is estimated that metal mine spoil heaps alone cover 16,490ha of land in the UK and the remediation progress has been much less effective than that achieved by the coal contamination. There is the additional contamination problem caused by water run-off from disused metal mines as water from the estimated 3,700 disused metal mines is entering rivers creating very damaging effects on plant, fish and animal ecosystems. The problem is not just the actual contaminated water but the fact that 90% of the metal contamination is in the sediments carried by the rivers. Flood plains are, therefore at great risk from sediment contamination. This pollution will continue for hundreds more years unless remedial action is taken. Research programmes around the world focus on two restoration methods listed below:

- Phytoremediation, which is directed at consolidating the spoil sites, uses plants, so that wind-blown contaminated soil and contaminated water movements are reduced.
- Phytoextraction, which is taking plant selection one step further by identifying plants that are able to extract heavy metals from the soil and tolerate unusually high levels of heavy metals within their cells.

High biomass plants could be very suited to phytoremediation and phytoextraction techniques in terms of the quantity of metals they could extract and store in their tissue. Novel planting technologies, such as the NEF CEEDS™ propagule technology, may also be very suitable when practically trying to establish plant populations on difficult terrain. On some heavy metal contaminated mine sites, there is evidence of natural 'remediation' as small clumps of grasses and small trees, which have clearly been established from natural seeding, manage to grow, but natural regeneration is not the solution.

4.4.5 Greenhouse Trials with Contaminated Land

The contaminated soils used in these trials were from two mines spoil areas, Bridford Barytes mine in Devon and Wheal Maid mine near Redruth in Cornwall. Five types of mine spoil from the two locations were initially used in the trials (Table 13: Elemental analysis of soils from different trial sites used to grow Miscanthus plants. Some of the levels of heavy metal contamination (mg/Kg) in the five mine soils expressed as a % of a typical agricultural soil were Sulphur 58,328%, Arsenic 32,654%, Lead 27,326%, and Copper 5,880%. These are extremely high levels of heavy metal contamination, and one was quickly discontinued after nothing could survive in the soil (Figure 90). Mine spoil was used in three pot trials where different treatments, such as a remediation product, fertiliser or microbes were also incorporated into the mine spoil. Plant performance was compared to that of plants grown in typical agricultural soil from the Taunton Trial location. All tissue analyses and soil analyses from Pot trials one and two were conducted by the sub-contractor, Plymouth University and Dr Robert Schindler's Report which is in Annex 4.

The principal findings from this analytical work were:

- Remediation products increased crop yield in a single mine waste soil in one greenhouse trial. However, it did not increase yield for the same soil, nor in the four additional mine waste soils. The weight of evidence suggests that the remediation product is largely ineffective in reducing the effects of toxicity on both crop yield and toxic element uptake.
- When remediation products are combined with Fertiliser, it increased crop yield and improved uptake compared with Control conditions.
- Microbes used in isolation did not cause a significant increase in crop yield or uptake of metals for contaminated soils. However, Microbes were effective when combined with Remediation and/or fertiliser.
- The combination of Remediation + Fertiliser + Microbes offered a synergy that both provided the largest crop yields and the highest amounts of toxic element uptake, regardless of the toxicity profile of the soil (Figure 91).
- Additives promoted increased uptake principally through uptake in roots rather than leaves. Only the combination of Remediation + Fertiliser + Microbes promoted increased uptake in both leaves and roots.
- These results suggest that roots filter and store toxic elements to an increasing degree when treated with additives, compared with Control conditions.

The two field trials, both of which were conducted at the Bridford mine, clearly demonstrated the profound effect of contaminated soils on plant establishment. The Control treatment in the first field trial had 25% plant survival after 450 days, the second field trial the Control had 17% plant survival after 150 days. However, the highest survival rate in Field Trial 1 was 55% from two treatments, both involving Remediation products and microbes. In Field Trial 2, it was 83% with just a remediation product.

Caution is necessary when interpreting this small database, but it is interesting that the fertiliser benefits noted in the pot trials do not appear in the field trials.

However, both trial approaches do suggest that there are avenues that can be pursued to remediate heavy metal contaminated soils and that phytoextraction was detected in these initial trials.

4.5 WP 5 Commercialisation

All figures listed in this section are provided in Annex 5.

4.5.1 Market Supply of Energy Crops

The number of planting material suppliers in the UK and the EU was assessed for different energy crops. EU suppliers for the UK market are also included as vegetative plant material can be imported to the UK without significant phytosanitary restriction, but the same material cannot logistically be exported from the UK to the EU. The market assessment showed that in the UK there are currently four providers of energy crop planting material, but only one of those has production in the UK, the others import all planting material from the EU to the UK. In the EU there are [redacted] the majority supply *Miscanthus* (90 %), with a small number of providers (10%) supplying *Arundo donax*, with no current suppliers of [redacted] in the EU.

The current planting capacity of the EU energy crop suppliers is estimated to not exceed 20,000 hectares (ha), without further onward propagation. The area in the UK of all types of energy crop is 133,000 ha, perennial energy crops (*Miscanthus* and willow) account for 9%, with Miscanthus contributing 6% of that with 8,800 ha.

4.5.2 UK and Export Market Demand for Energy Crops

For electrical generation, four UK power stations consume an estimated 1,000,000 tonnes of straw annually, of which around only 3% (30,000 tonnes) is currently *Miscanthus*, the rest from cereal straws. However, the retention or expansion of this figure may change, as some end users are reconsidering the use of *Miscanthus* depending on the continuation of Renewable Obligation Certificates (ROCs) after 2027, which is a key support mechanism for renewable energy

UK forward demand for energy crops in the most recent UK Biomass Strategy document[10] projects energy crop plantings rising between 9,000 to 17,000 ha per annum by 2038. Other policy support[11] projects the area rising to 700,000 ha by 2050. This would require 3% of UK farm-land area but would represent a transition away from imports to using mainly UK feedstock of energy crops and biomass residues[12].

These forward market assumptions create a low, medium and high expansion of dedicated energy crops in the UK of 214,000, 435,000 and 700,000 ha respectively. When assessed at a current yield in the range of 10 t ha^{-1}, and forward increases in yield of 15 and 20t ha^{-1}, this shows an energy crop supply capability between 2-14 million tonnes per annum (Figure 95). These supplies are between 2-14 times higher than feedstock offtake into installed generating capacity in the UK. For these forward energy crop planting scenarios to be met a significant investment in installed generation capacity in the UK or the development of alternative markets would need to be made. All projections also require a step change in planting, with less than

[10] https://assets.publishing.service.gov.uk/media/64dc8d3960d123000d32c602/biomass-strategy-2023.pdf
[11] https://www.theccc.org.uk/wp-content/uploads/2025/02/The-Seventh-Carbon-Budget.pdf

10,000 ha planted in the last 20 years (and under 9,000 ha now remaining) the area of current *Miscanthus* planting or double this total is required to be planted annually. Current planting resources in the UK and EU represent only 2-10 % of this capacity respectively, so new resources, varieties and systems are required for this scale-up of UK energy cropping. In addition, the UK the government plans to re wet large areas of peat soils in the UK. There are 325,000 ha of lowland peat soils in the UK which hold over 50% of the total terrestrial stored carbon, but less than 1% of them remain in a natural state[13]. 74% of them are used for farming, and the UK government plans to potentially rewet around 100,000 ha[14] and farm it is using wetland plants such as reeds and grasses, named paludiculture[15]. Using plants such as *Typha* which restore this land, sequester carbon and offtake significant amounts of phosphate from the water. Current suppliers of these plants in the UK are typically for small-scale planting; larger-scale capacity with lower-cost planting propagules is required to meet this market demand.

4.5.3 Commercial Supply Models

This project has developed two main commercial supply models to meet the demand highlighted above. The first is varietal licensing of new energy crop varieties developed in this project, so they can be used by growers to increase yield capability and also provide Intellectual Property (IP) protection and royalty collection to the breeders. Secondly, commercial propagation solutions have been developed with NEF CEEDS™ and other technologies to allow larger, faster and better propagation and planting of these crops. Providing solutions for both arable land, degraded contaminated land and paludiculture on wetlands.

Varietal Licensing

The varieties are perennial energy crops, in contrast to the majority of annual crops where yearly replating results in regular income for breeders. The principal of energy crops is that once established, annual costs are lower. The chosen commercial model has been for an annual royalty paid at planting to avoid issues of multi-year collection. This amount would be proportional to any benefit, so for example, a variety that provides cold tolerance and is the only one that can be grown with certainty in a specific region can justify a royalty. The royalty rate specifics have been negotiated with the breeders, with a focus on simpler annual payments. NEF revenue is an agreed % of the royalty income, as the breeder alone cannot ramp up the volumes of material, sell them or collect from growers. However, under most scenarios it is envisaged that customers would be licensing one or more propagation technologies and multiple varieties. A licensing agreement for varieties has been developed for licensing out to customers, the protects the IP of the breeders in allowing users to grow their variety. NEF also have an inward licence from the breeder (provided by them). This agreement is drafted so that specific terms can be added to reflect these agreements.

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[13] DEFRA UK 2023. https://www.gov.uk/government/news/new-investment-in-peat-in-fight-against-climate-change
[14] https://www.wwt.org.uk/uploads/documents/2024-10-17/100000-hectares-of-uk-wetlands-exploring-the-potential-wwt-1710.pdf
[15] https://lowlandpeat.ceh.ac.uk/sites/default/files/2022-07/Defra-LP2-paludiculture-report-April-2020.pdf

Propagation Technology Licensing

The CEEDS™ propagation system is based on a bio-factory model, outlined in more detail in Background of the main report and Annex 2. Compared to field propagation, which uses extensive production over a large area of field production, CEEDS™ bio-factories are small-scale horticultural units that propagate crops using intensive modern systems with a smaller production footprint. Smaller-scale production systems (and plants) also support greater automation of the system. The bio-factory is divided into four areas, which are expanded on in more detail in Background of the main report and Annex 2. The bio-factory units can be set up for different volumes of production. In this project a model with an annual planting output of 1,000 ha was established. This is based on the UK supply scenarios, where 7,000 ha yr^{-1} upwards of new energy crop planting is expected. Multiple regional CEEDS™ bio-factories could be established in regions where high levels of plantings were expected, to meet this demand. The production area and sizes below, are based on this model of multiple 1,000 ha units.

Overall production space requirements for CEEDS™ bio-factories for 1,000 ha of output vary between 5.4 to 5.9 ha for rhizome (Figure 96) and stem (Figure 97) systems respectively. This space requirement is broken down into the four areas of nodal production, hardening, greenhouse and processing buildings. Compared to field propagation of rhizome and stem crops, the CEEDS™ system represents a significant reduction in production area required. Compared to rhizome propagation, which requires circa 55 ha of field rhizomes to plant 1,000 ha, a CEEDS™ bio factory can reduce this space by 10-fold to less than 5.5 ha (Figure 96). For stem propagation, the field production space is higher, with 188 ha required for 1,000 ha of replanting. A CEEDS™ bio factory can reduce this space by 31-fold to less than 6ha (Figure 97). This reduced scale for propagation capacity, if scaled to full market supply in the UK of 7,000 ha yr^{-1} planting, reduces the propagation space requirement from 385 ha using conventional field production to 39 ha using the CEEDS™ bio-factory model.

For licensing, a flexible model was required to support the commercialisation of CEEDS™ and priming propagation technology. A licensing model was preferred to internal production as a large proportion of the potential customer base favours internal production for themselves (vertically integrated users) and another section (distributors) has existing customer-facing investments to distribute. The flexible model chosen was a licensing structure that can be applied to all three situations. This supports either a large farming customer licensing, a distributor, or a vertically integrated end user, which should meet the requirements of all customers. Two propagation products are being licensed. Firstly, the CEEDS™ system (Crop Expansion, Encapsulation and Delivery System). This is an artificial seed system where bio-factories are set up as new faculties (which are intensive horticultural sites). The CEEDS™ system can be applied to any perennial grass type. Secondly, is the priming system which is a treatment system to enhance the germination and vigour of rhizomes. This system is currently specific to *Miscanthus* crops and intended as an-add on system to existing rhizome processing operations (to treat rhizomes after lifting from the ground). Legal licensing agreements have been created for CEEDS™ and priming. All agreements have been developed so they can be used for either of the propagation types, and in each case, be easily modified to add in specifics, to remove the need for new agreements with each licensing customer. For the licensing of propagation solutions developed for the paludiculture market, it has not been determined so far if these are going to be fully out licensed, as with the CEEDS™ system for energy crops. The current plan is initially for internal production of planting material using these systems, with a further review to see if this can be repeated for further expansion or if the licensing model will be adapted. These systems are smaller scale and require less capital, so internal production is an option.

To support the evaluation of the platform cost, models have been prepared by NEF for the two types of energy crop, those that propagate via either rhizome or stem more easily. Rhizome crops refer to species such as *Miscanthus* that are propagated from root, below ground material, whereas stem energy crops refer to types such as the energy cane (related to sugarcane) that are propagated from stem, above ground material. This has allowed a range of scenarios to be modelled. All are for 1,000 ha of planting output per year: High (H), Base case (BCC) and Low options (L) for both rhizome and stem models the three high. All models also have to speed up the ramp of propagation material, resulting in 12 scenarios modelled overall. The High (H), Base case (BCC) and Low (L) options vary in a number of factors between scenarios. The factors include components such as the cost of labour, installation cost and propagation efficiency.

Total CAPEX projections for the establishment of a 1,000 ha CEEDS™ bio-factory [redacted]. Cost projections are based on different levels of operating efficiency and equipment costing. The percentage contribution to the overall capital cost shows that the greatest contributor is the site buildings at just under 40% of total CAPEX. When evaluated on a site-by-site basis, there may be existing building that can be used directly or modified at a lower cost than new building construction.

Total operating projections for the establishment of a 1,000 ha CEEDS™ bio-factory are based on different levels of operating efficiency and input costings. The percentage shows that the greatest contributor is the seasonal labour required for production, followed by tissue culture plants. Tissue culture plant costs are higher under scenarios with only one multiplication step before hardening. This is also reflected in the COG projections which also show the double multiplication stage is optimum for cost reduction.

Projected costs of goods (COG) are based on 1,000 ha planting output per annum site, operating for 13 years, which covers a 3-year ramp up to full capacity, followed by ten years of steady-state operation. [redacted]. The range in costs can be attributed to a wide range of operating values, such as labour costs, between the different production regions. These costs represent reductions in the production cost compared to conventional production systems.

4.5.4 Commercial Licensing Package

For commercial licensing, six components have been produced to provide the required information to prospective customers and then deliver the technology transfer during licensing:

- Licence
- Financial model
- Processing equipment and layout drawings

- Field planting equipment
- Production protocols
- Marketing materials

Full layout drawings have now been produced for the two variants of the bio-factory for stem and rhizome propagation. These have numbered machinery, which is all coded. All equipment has supporting information on its capital cost, usage of inputs (power, water and materials such as substrate or coating equipment), throughout assumptions, maintenance and labour requirement. This information is in a data book, which is used to populate the financial model. These CAD drawings are of sufficient detail to be used to draw up local design documents for a CEEDS™ licensing customer to implement. The required processing buildings have been designed and specified to allow accurate costing, with drawings complete sufficient to implement construction.

Field mechanisation of planting for CEEDS™ has been developed in the project. Further details on the development of this machinery provided in Annex 2 and images of the planting developed and built in this project are given in Figure 98. The planter was made in partnership with a commercial agricultural engineering company (Standen Engineering). This allowed for more rapid development of the equipment, with their engineering expertise, but also has established a platform for commercial supply. Planters can now be manufactured, sold and supported in the UK to support forward scale-up.

In terms of commercial use and contribution to sustainable biomass supply in the UK, the development of this planter provides essential capacity building. A CEEDS™ bio-factory model developed in this project is for units capable of producing 1,000 ha of energy crop planting per year. In terms of planting, current *Miscanthus* production systems use two options: manual (precision) and automatic rhizome planters. These have a planting capacity of approximately 0.5-3ha per hour respectively. The precision planting approach is favoured and works well for current plantings, which are typically in the range of 10ha fields, but it is slow and uses high amounts of labour (Figure 99). The automatic planter can deliver higher planting rates and is suited to situations where higher volumes of planting material are used per ha of planting. The precision planting route is favoured by customers, as it provides a more uniform establishment. However, 1,000 ha of spring planting using precision planters, based on a planting window of 42 days (which is the optimum window) and an operating efficiency of 75%, gives a planting window of 31 days. To deliver this using current precision rhizome planter would require nine planters operating, circa nine tractors and five telehandlers (Figure 100) and 38 people (Figure 99).

Using automatic CEEDS™ planters, these have an output of 1.5 to 3.0 ha hr^{-1} for 2- and 4-row variants, respectively. A 2-row planter has been developed and built in this project to test the concept. The design used is modular, with the expectation that 4-row machines will be the commercial optimum for the UK. The 4-row CEEDS™ planter delivers the same speed or higher as automatic planters, but with precision planting six times faster than current (precision planting) rhizome systems. When applied to the 1,000 ha yr^{-1} planting scenario (Figure 99 & Figure 100) this shows that planting via CEEDS™ can deliver the same planting area using one planter compared to nine with current precision planters and using three people, not 28. This option delivers a scalable precision planting system, that can deliver the higher areas of planting required (9,000 to 17,000 ha yr^{-1}). Expansion of manual planting to these levels would be logistically difficult given the number of staff required, combined with the low operating efficiency of spring planting with any system. There are also additional benefits for the CEEDS™ planter beyond planting speed, in terms of safety, the ability to integrate minimum tillage systems and a higher percentage of the seasonal planting to occur in the optimum planting window (to maximise first year yield).

Full written production protocols have been produced for the rhizome and stem production systems. For each process, stem or rhizome there are 34 individual protocols, which are divided into five sections that cover all parts of the process through to field planting. Each individual protocol has step-by-step instructions with images to train licensees in the process. Marketing literature has been produced specifically for the project, to update information on the licensed products of propagation technology and energy crop varieties. The material is staged with initial formal outline information on NEF, the propagation technology and energy crop varieties. Additional information is modified specific for customers, covering project-specific information to the customer. This includes specific financial proposals covering capital cost, operating cost and space requirements, plus licensing details and a technology transfer proposal. For varieties, further information is site-specific yield data where available, and feedstock quality data for different end markets from combustion to digestion uses.

5. Contribution to Sustainable UK Biomass Supply

Miscanthus is identified as a key crop for the UK to develop a sustainable biomass supply. The current yield of *Miscanthus* is projected at 12t ha^{-1} yr^{-1}, increasing to 20 t/ha by 2050[16]. 12t ha^{-1} is in line with current yields[17,18,19] and provides a solid return for landowners, but it has not stimulated more than 10,000 ha of commercial planting of *Miscanthus* crops in the UK. This has been delivered using one non-improved sterile, vegetative *Miscanthus* variety. Increasing yield, rather than lowering the cost of establishment, is the main agronomic factor that raises the economic return to farmers, delivering the step change required to increase planting. Investment in *Miscanthus* breeding over the last 15 years in the US, EU and UK has mostly focused not on yield but on developing a new type of establishment, using seeded varieties drilled like cereals. However, it has now been concluded that seeded hybrids of *Miscanthus* cannot be directly drilled but must be grown in a greenhouse before being planted as plug plants[20], resulting in costs equal to current rhizome production. In addition, seeded hybrids have not increased yields and the average trial yield has been less than the 12t commercial

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[16] https://www.theccc.org.uk/publication/the-seventh-carbon-budget/

[17] Extending *Miscanthus* Cultivation with Novel Germplasm at Six Contrasting Sites. Front. Plant Sci. 8:563. Kalinina O *et. al.* 2017.

[18] Economic and Environmental Assessment of Seed and Rhizome Propagated *Miscanthus* in the UK. Front. Plant Sci. 8:1058. Hastings et. al. 2017.

[19] Breeding strategies to improve *Miscanthus* as a sustainable source of biomass for bioenergy and biorenewable products. Agronomy, 9(11), 1-17. Clifton Brown, J. *et. al.* 2019.

[20] Wu, PC, Ashman, C, Awty-Carroll, D, Robson, P and Clifton-Brown, J (2022) Optimizing seed-based *Miscanthus* plug plant production with supplemental heat and light, compost type and volume. GCB Bioenergy, 14 (6). pp. 624-638. ISSN 1757-1693.

baseline yield[21,22,23]. Seeded hybrids are fertile and could also encounter barriers to larger-scale planting due to invasive concerns. This project has focused on delivering a significant beneficial improvement to the UK biomass supply by identifying new, higher yielding, sterile, energy grass varieties for growers to plant in the near future.

This project harnessed the global breeding of *Miscanthus* and other suitable energy grasses for the UK in order to remove the reliance on just the UK *Miscanthus* seeded hybrid breeding programme and to deliver yield increases. NEF was able to source a total of 27 varieties of *Miscanthus* and other energy grass crops, which have high yield potential. In tests outside the UK, the candidate varieties had reported yields ranging from 34 t/ha to 60 t/ha from the best *Miscanthus* and cold-tolerant sugarcane types respectively.

UK forward demand for energy crops in the most recent UK Biomass Strategy document[24] projects energy crop plantings rising between 9,000 to 17,000 ha yr^{-1} by 2038. Other policy support[25] projects the area rising to 700,000 ha by 2050. These forward market assumptions create a low, medium and high expansion of dedicated energy crops in the UK of 214,000, 435,000 and 700,000 ha respectively. For these areas to be planted by 2025, 9,000, 17,000, and 30,000 ha would be required to be planted per annum. The output from this project can make the following contributions to sustainable UK biomass supply.

- Increases in yield would support a greater biomass production on the same land area, which at the highest planting projection could produce 14 million tonnes of biomass per year, compared to current projections of 8.4 million tonnes dry matter (Figure 6).
- The propagation area required would also be ten times lower if using CEEDS™ bio factories compared to conventional field rhizome propagation (Figure 7). At the highest planting projection this would need 158 ha compared to 1,650 ha (Figure 7).
- For planting, CEEDS™ precision planters can plant as fast as automatic planters but deliver precision planting that can currently only be delivered by manual planters. The impact of this is that at the highest planting projection the number of machines required would be 90 (including 30 planter), compared to 690 with conventional precision planters (composed of 210 planting machines, tractors and farm handlers). In addition, the people requirement would also be reduced from 1,140 to 90 (Figure 8).
- Five to ten new energy crop varieties will be available to UK farmers.

These contributions can potentially increase the biomass supply using less land and have a greater chance of success with an increase in landowner or farmer income with higher yield. Planting logistics are also improved, which reduces the risk of not completing planting in the spring with faster planting and fewer people.

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[21] Extending *Miscanthus* Cultivation with Novel Germplasm at Six Contrasting Sites. Front. Plant Sci. 8:563. Kalinina O *et. al*. 2017.
[22] Economic and Environmental Assessment of Seed and Rhizome Propagated *Miscanthus* in the UK. Front. Plant Sci. 8:1058. Hastings et. al. 2017.
[23] Breeding strategies to improve *Miscanthus* as a sustainable source of biomass for bioenergy and biorenewable products. Agronomy, 9(11), 1-17. Clifton Brown, J. *et. al*. 2019.
[24] https://assets.publishing.service.gov.uk/media/64dc8d3960d123000d32c602/biomass-strategy-2023.pdf
[25] https://www.theccc.org.uk/publication/sixth-carbon-budget/

Beyond farmland this project has also developed solutions for producing planting propagule for wetland remediation, either for enhanced carbon sequestration or combined with additional feedstock production. On contaminated land, the project has identified how *Miscanthus* can be established on land previously not considered suitable for biomass production, which could release further feedstock potential and at the same time, remediate non-productive land.

Figure 6: *Projected peak biomass supply in 2050 under three planting scenarios of 214,000, 435,000, and 700,000ha of energy crops, with different peak yield values of tonnes per ha per year of dry matter.*

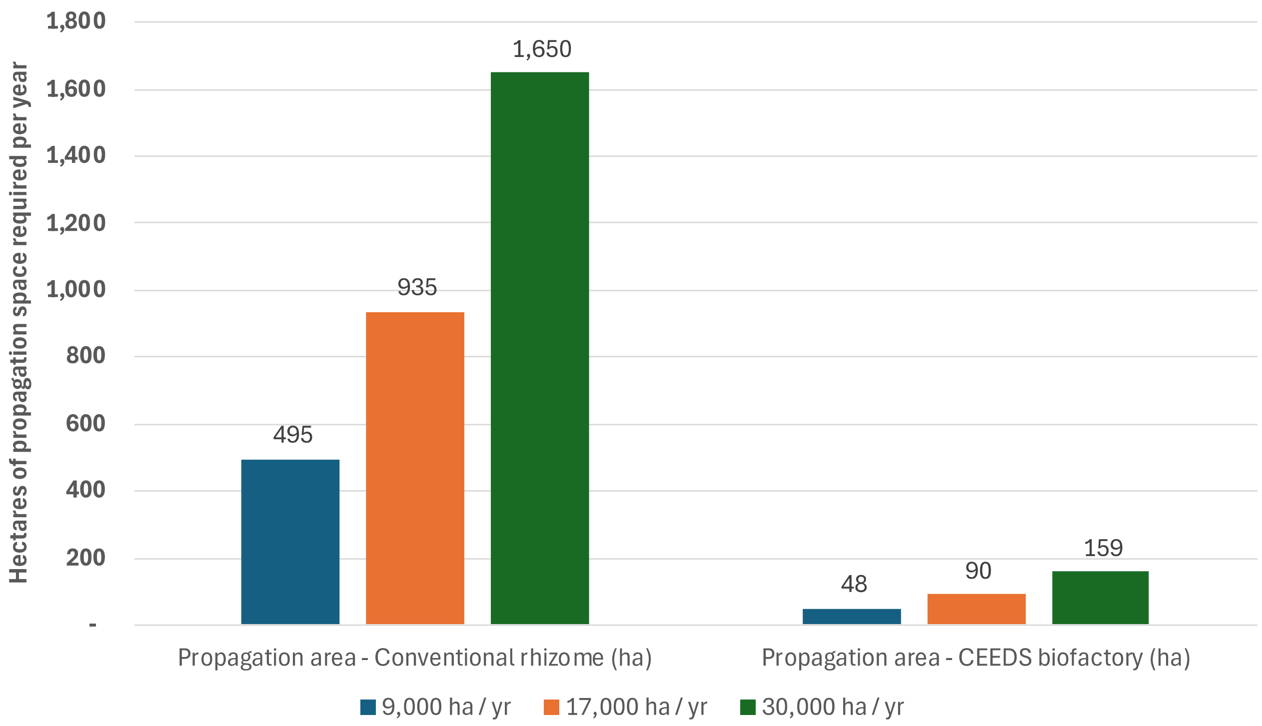

Figure 7: *Area (ha) of propagation space required under three planting scenarios of 9,000, 17,000, and 30,000ha of energy crops per year, using either conventional field propagation of Miscanthus via rhizomes, or using CEEDS™ bio factories.*

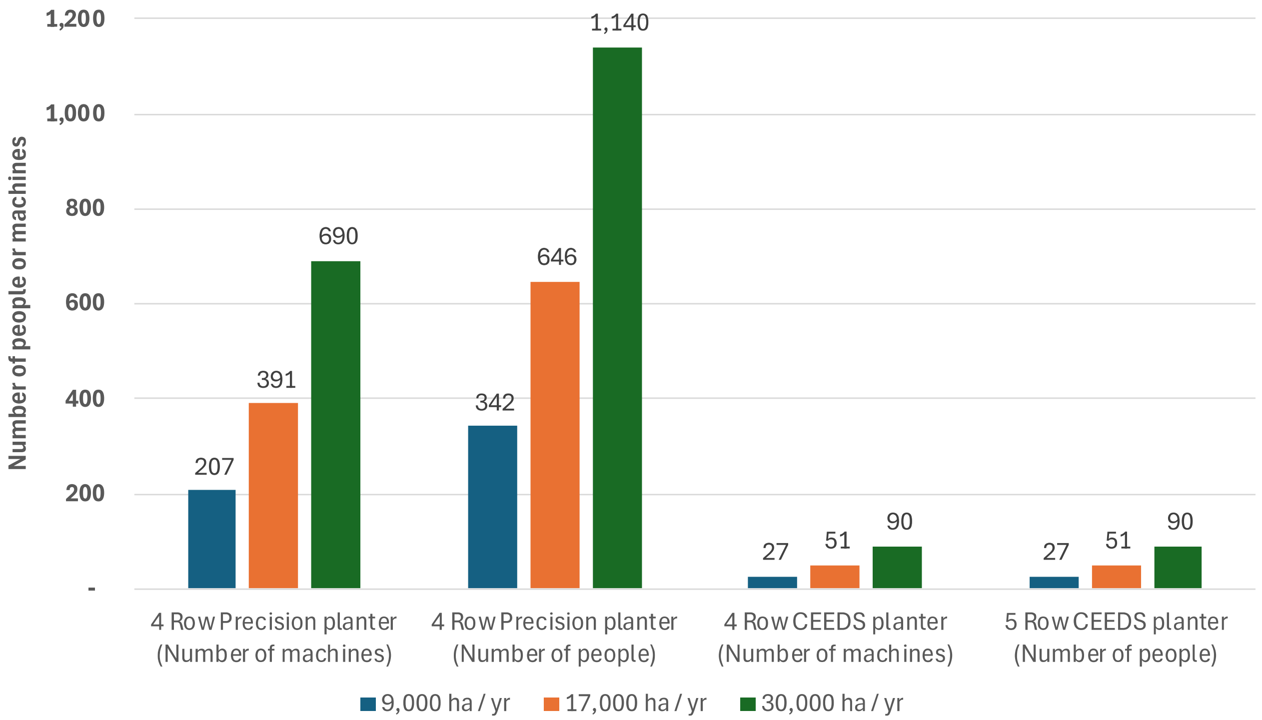

Figure 8: *Number of machines and people required under three planting scenarios of 9,000, 17,000, and 30,000ha of energy crops per year, using either conventional manual precision planters for Miscanthus via rhizomes, or using CEEDS™ automatic planters.*

6. Key Successes

- A diverse range of new energy grasses were imported and tested for the first time in the UK at five sites around the UK, using field testing sites. Results showed that six varieties could outperform the control of current *Miscanthus* types. With projected peak yields in the range of 16.8 to 29.5 t ha^{-1} yr^{-1} dry matter, based on extrapolation to current field planting densities. The trial sites are being continued as other materials imported into the project also show greater potential.
- Propagation systems were successfully developed based on NEF's CEEDS™ system for a range of high-yielding energy grasses. The work covered all aspects of production from initial propagation through to coating to make the final energy crop CEEDS™. Field planters were developed and constructed.
- Microbial work completed the most detailed assessment of UK *Miscanthus* crops, aged 2-27 years of age. The results showed significant differences in fungal populations between sites, but similarity of bacterial populations was not expected.
- New propagation and novel planting solutions were developed for planting the paludiculture crop *Typha*.
- For contaminated land, it was demonstrated how combinations of chemical treatments could support the establishment of *Miscanthus* on land highly contaminated with heavy metals.
- These outputs were developed into a commercial bio-factory model for CEEDS™ production, an intensive horticultural site that can produce 1,000 ha yr^{-1} of planting material. Using a production footprint 10-20 times lower than conventional field propagation technology combined with fully automatic CEEDS™ planters. Business model licensing was established as the commercialisation route following project completion.

7. Persistent Barriers

[redacted].

A second issue is the installation of sufficient off-take capacity in the UK. The forward market assumptions outlined in Section 5.5 create a low, medium and high energy crop supply capability between 2-14 million tonnes yr^{-1}. These supplies are between 2-14 times higher than feedstock offtake into currently installed generating capacity in the UK. For these forward energy crop planting scenarios to be met there would need to be significant investment in installed generation capacity in the UK or development of alternative markets.

8. Impact of Innovation on Greenhouse Gas Emissions

A Life Cycle Analysis (LCA) model was developed to estimate the greenhouse gas emissions (GHG) emissions projected from CEEDS™ production. They can then be compared to other forms of propagation. The model takes account of the primary energy inputs from electricity and liquid fuels, plus all consumables used from substrate to end-use packaging. The model shows a GHG production cost for CEEDS™ (Figure 9) of 129.6 Kg of CO_2 ha^{-1} of crop produced, plus an additional 263 Kg to plant the crop, allowing for all field operations and applied fertilizer and crop protection. This results in a total per ha planted of 392 Kg of CO_2 ha^{-1}. Independent work on *Miscanthus*[26] using other establishment methods of stem cuttings[27] and rhizome cuttings[28], has projected GHG emissions of between 809 to 1,151 Kg of CO_2 ha^{-1} for stems and 455 – 2,118 Kg of CO_2 ha^{-1} for rhizomes (Figure 10). Based on these figures, the CEEDS™ establishment system would have lower GHG gas emissions than current production systems.

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[26] McCalmont, J., Hastings, A., Mcnamara, N., Richter, G. M., Robson, P., Donnison, I., & Clifton-Brown, J. (2017).Environmental costs and benefits of growing Miscanthus for bioenergy in the UK. GCB Bioenergy, 9(3), 489-507.
[27] O'Loughlin et. al. Quantifying the economic and greenhouse gas balance advantages of establishing Miscanthus from stem cuttings. Biomass and Bioenergy 109 (2018) 147–154.
[28] Hastings et. al The technical potential of Great Britain to produceligno-cellulosic biomass for bioenergy in current andfuture climates. GCB Bioenergy (2014) 6, 108–122, doi: 10.1111/gcbb.12103.

The additional benefit is that CEEDS™ can be applied to potentially higher-yielding energy grasses. Sometimes, these will be *Miscanthus* rhizome-based types, but they will require stem propagation in other situations. Under UK conditions, stem propagation is not a viable option, given the lower temperature. CEEDS™ can use either stem or rhizomes as the propagation material. The UK GHG cost of harvesting *Miscanthus* is 57 Kg of CO_2 per tonne of material. So, when the establishment cost is amortized over ten years, the annual total CO_2 cost per ha can be calculated at either 10-25 t ha^{-1} a forward high yield assumption. On a per ha basis, there is less sensitivity to the GHG cost of establishment, compared to greater sensitivity if the harvesting GHG cost could be reduced, which is a greater contributor to emissions per ha. When expressed on a per tonne basis (Figure 11) this is reinforced but does show that the lowest GHG cost is delivered via CEEDS™ at 59 Kg of CO_2 t^{-1}, compared to 78 Kg of CO_2 ha^{-1} with a rhizome crop at 25t ha^{-1} yield. This represents a 25% reduction in GHG per tonne using CEEDS™ as a benefit of this project.

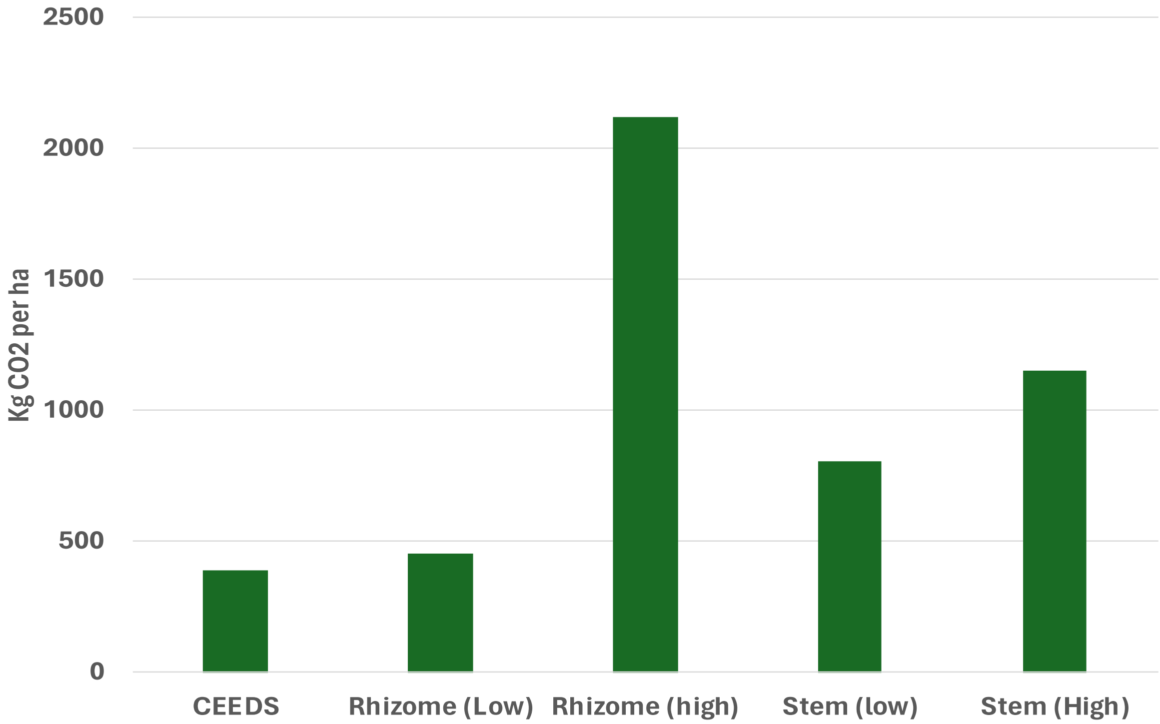

Figure 9: *CHG intensity (Kg of CO2 per ha) to establish a crop of Miscanthus using different planting technologies.*

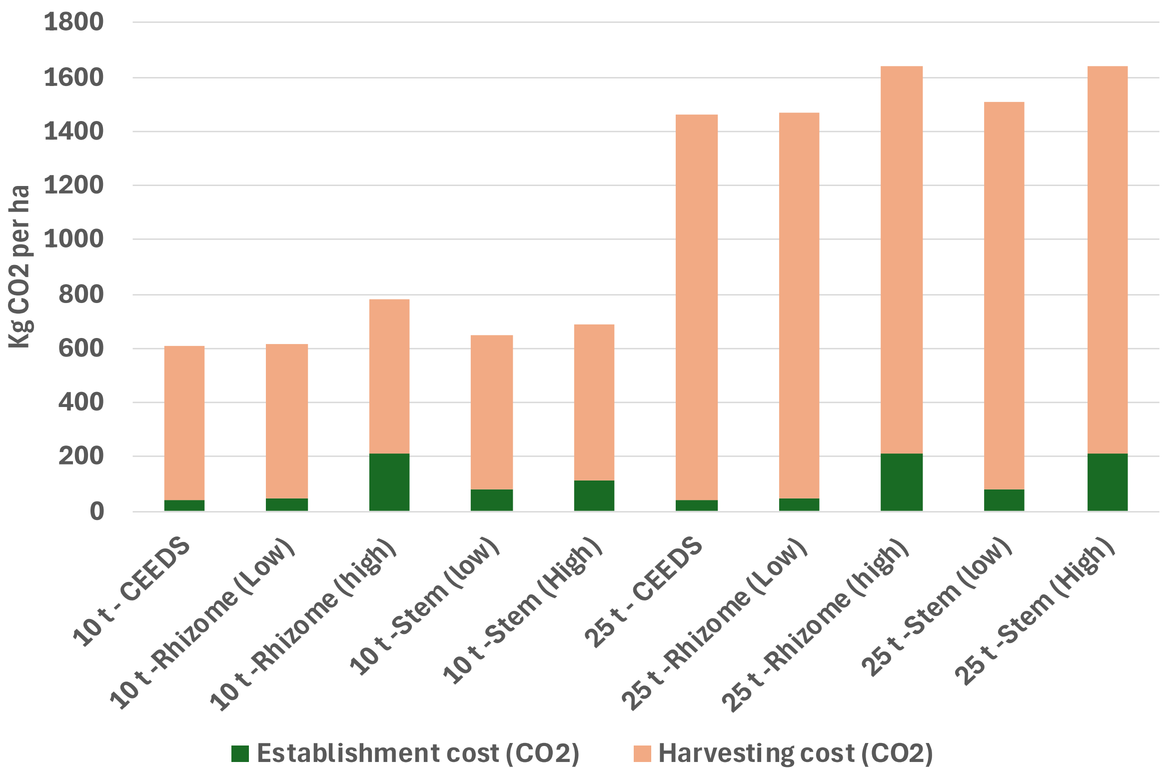

Figure 10: *CHG intensity (Kg of CO2 per ha per year) year for a crop of Miscanthus using different planting technologies and different dry matter yields per ha of 10t or 25t. The GHG cost of establishment has been amortized over ten years.*

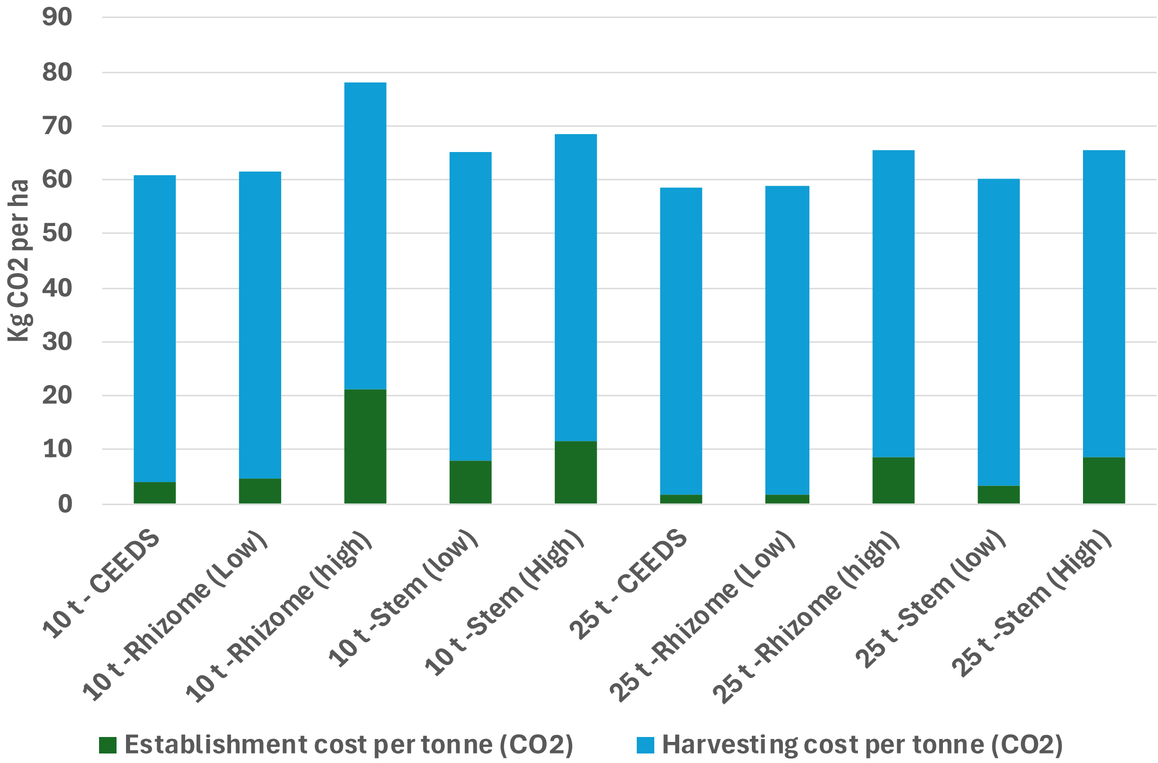

Figure 11: *CHG intensity (Kg of CO2 per tonne dry matter of biomass harvested to bales) year for a crop of Miscanthus using different planting technologies and different dry matter yields per ha of 10t or 25t. GHG establishment cost amortized over ten years and harvesting cost to bales per tonne.*

9. Commercialisation

As outlined in Section 4.5 the project has developed a commercial package, based on the outputs from this project. These cover three main areas of:

- New energy crop varieties.
- Propagation technology licensing, CEEDS™.
- Novel propagation technology developed for the paludiculture markets.

New Energy Crop Varieties: A package has been developed with yield and performance data on varieties new to the UK market, also covering licensing agreements. The first commercial licence for one of these varieties has been secured and will commence after the completion of the project.

Propagation Technology Licensing: The licensing package has been promoted, and a pipeline of commercial customers has been established in the EU, North America, South America and other regions. The first commercial licence for propagation technology licensing has been agreed and will commence after the completion of the project.

Novel Propagation Technology Developed for the Paludiculture Markets: The new technology that has been developed for the paludiculture market is currently being evaluated for internal production by NEF in the UK, to develop a commercial supply capability. In regions beyond the UK, a licensing model is likely to be followed.

10. Secondary Project Benefits

The project directly created eight new roles over the three-year period with staff recruited for their expertise and skills within the Southwest Region. Six of these staff worked directly on the plant research side, with two project specific roles in project management and financial modelling. The staff have all benefitted from developing their technical and commercial experience and skills during the three years and remained to the end of the project. Alongside formal qualifications, staff have developed specialist knowledge which they are all now taking forward into plant technology research roles.

NEF secured two further large commercial projects which three of the employees moved into after the project closed. Other members of team were successfully recruited into similar roles with a local commercial licensee, where they will continue to grow and develop their expertise.

Through the use of local Subcontractors and Casual labour (around harvest and planting time), the project has greatly supported the local economy. This local expertise of Subcontractors has been key in developing the custom-made machinery designed around the planting technology.

Two of the National subcontractors were Universities, where PhD students have been able to develop their own level of knowledge and expertise on the planting material and methods. The project has resulted in three new research projects being developed for which funding is currently under review for, to continue the work from this project.

Farmers nationally have benefitted from the land rental, hosting the trial sites and will be maintaining these relationships with them to continue to evaluate the field trials areas.

A wide range of small to medium sized companies have also been involved in the supply chain, either in the sale of materials to support the plant research or engineering and machinery supplies for the CEEDS™ technology.

11. Project Management

The project was split into the five work packages detailed above with Dr Mike Carver and Dr Paul Carver overseeing the technical work. Activities were scheduled to coincide with the lifecycle of the plants, and the building and testing of the technology. Local external agencies and labour markets when used to cope when the demand for labour was high (i.e. planting and harvesting).

Our project Manager was responsible for overseeing the schedule of work and managing the finances of the project. Budgets were allocated at the start of the project back in 2022, these have stayed in line with forecast figures but in many instances have been broken down into smaller milestones in order to help with cash flow.

12. Conclusions

- A diverse range of new energy grasses were imported and tested for the first time in the UK at five sites around the UK. Results showed that six varieties could outperform the control of current *Miscanthus* types.
- Projected peak yields of these new grasses were in the range of 16.8 - 29.5 t ha^{-1} yr^{-1} of dry matter based on extrapolation to current field planting densities, compared to typical yields in the range of 10-15 t ha^{-1} yr^{-1}.
- The trial sites are being continued as other materials imported into the project also show greater potential.
- Propagation systems were successfully developed based on NEF's CEEDS™ system for a range of high-yielding energy grasses. The work covered production from initial propagation through to coating to make the final energy crop CEEDS™.
- New CEEDS™ field planters were developed and constructed.
- Microbial work completed the most detailed assessment of microbes in UK *Miscanthus* crops, aged 2-27 years of age. The results showed significant differences in fungal populations between sites, but similarity of bacterial populations was not expected and had not been reported before.

- New propagation and novel planting solutions were developed for planting the paludiculture crop *Typha*.
- For contaminated land, it was demonstrated how combinations of chemical treatments could support the establishment of *Miscanthus* on land highly contaminated with heavy metals.
- A commercial bio-factory model for CEEDS™ production, an intensive horticultural site that can produce 1,000 ha yr^{-1} of planting material.
- Using a production footprint 10-20 times lower than conventional field propagation technology combined with fully automatic CEEDS™ planters. Business model licensing was established as the commercialisation route following project completion.
- This work was based on the potential increase in UK energy crop 700,000 ha by 2050. Increases in yield from these new varieties would support greater biomass production, with the same land area capable of producing up to 14 million tonnes of dry matter, compared to current projections of 8.4 million tonnes.
- Planting these crops from 2026 onwards at a rate of 30,000 ha yr^{-1} would currently require a propagation area of 1,650 ha. This requirement could be reduced tenfold using CEEDS™ bio-factories.
- Annual planting at this scale using CEEDS™ precision planters would require just 30 planters, compared to 210 manual planters currently needed.
- Beyond conventional farmland, this project also developed solutions for wetland remediation and previously unsuitable land, unlocking additional feedstock potential while contributing to the remediation of non-productive land.
- A Life Cycle Analysis was conducted for the propagation systems developed in the project. It showed that GHG emissions could be reduced by 14-18% compared to the baseline emissions associated with establishing *Miscanthus* from rhizomes in the UK.

Annexes

Annex 1 – Supporting information for WP 1, Varieties

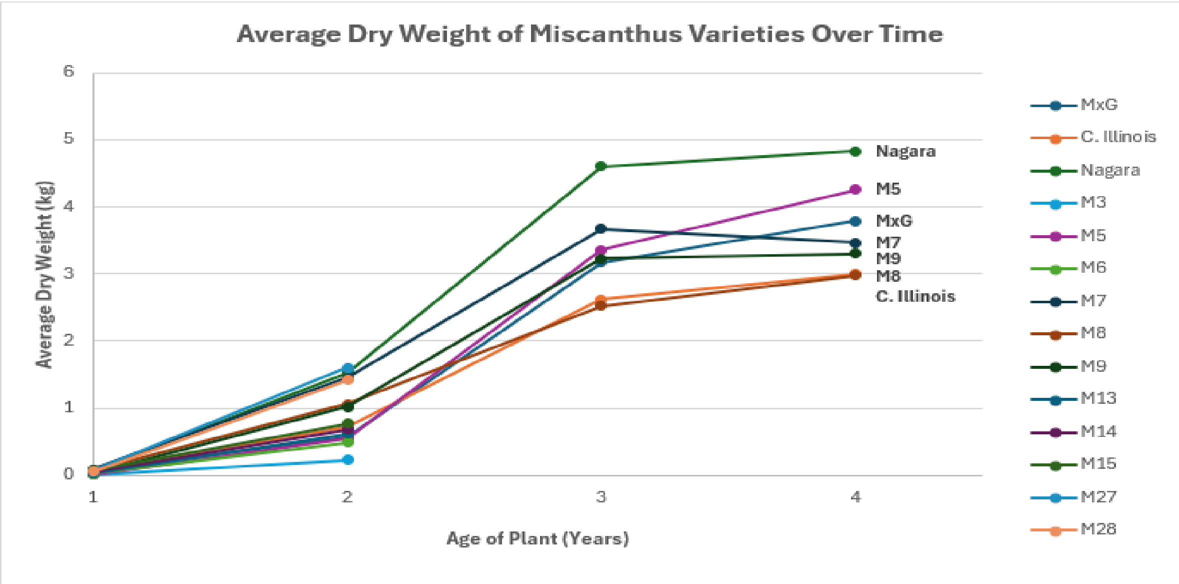

Figure 12: *Average dry weight (Kg) of seven Miscanthus varieties over time from specimen plots.*

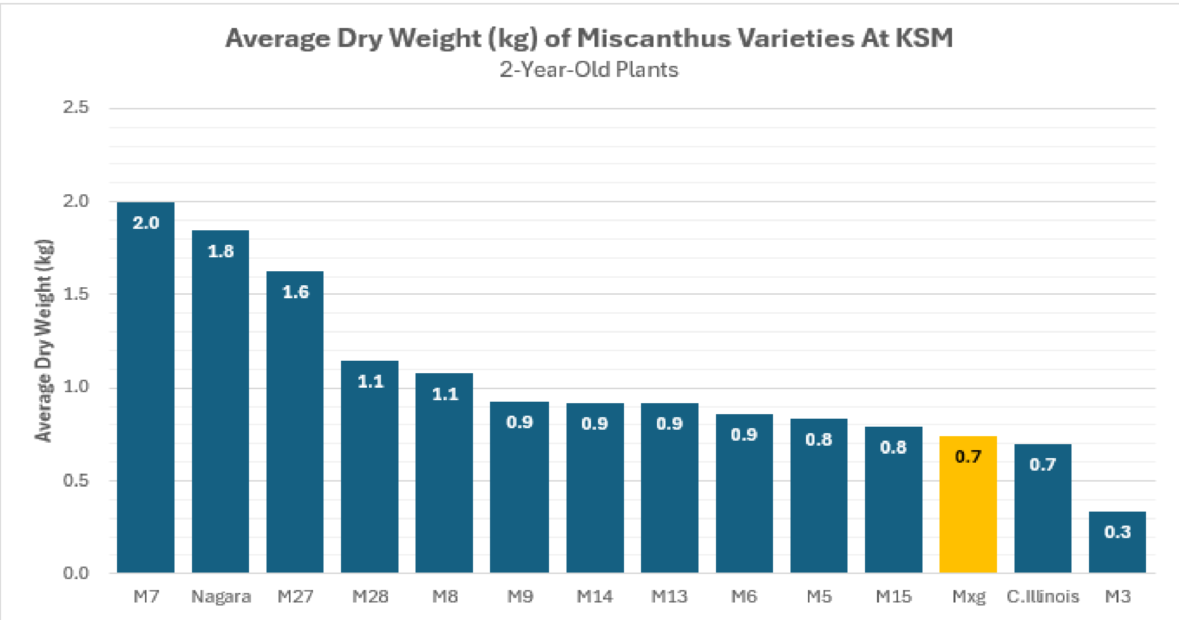

Figure 13: *Miscanthus dry weight plant yields, from specimen plots, Taunton harvest, 2025.*

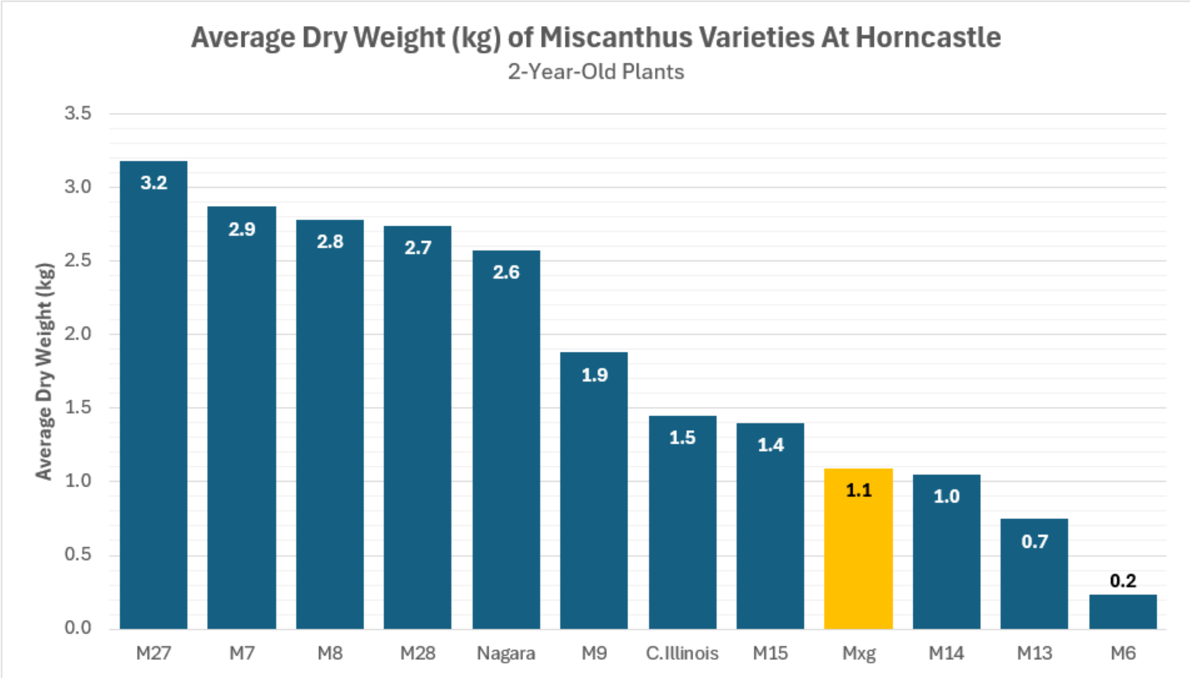

Figure 14: *Miscanthus dry weight plant yields, from specimen plots, Horncastle harvest 2025.*

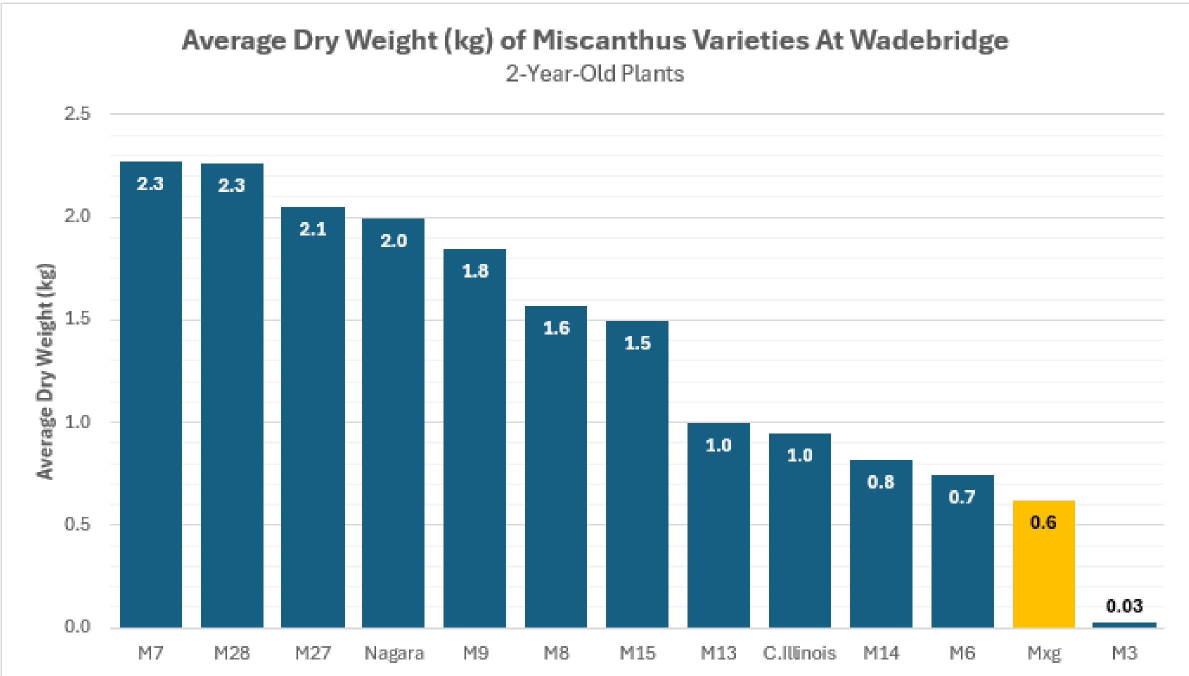

Figure 15: *Miscanthus dry weight plant yields, from specimen plots, Wadebridge harvest 2025.*

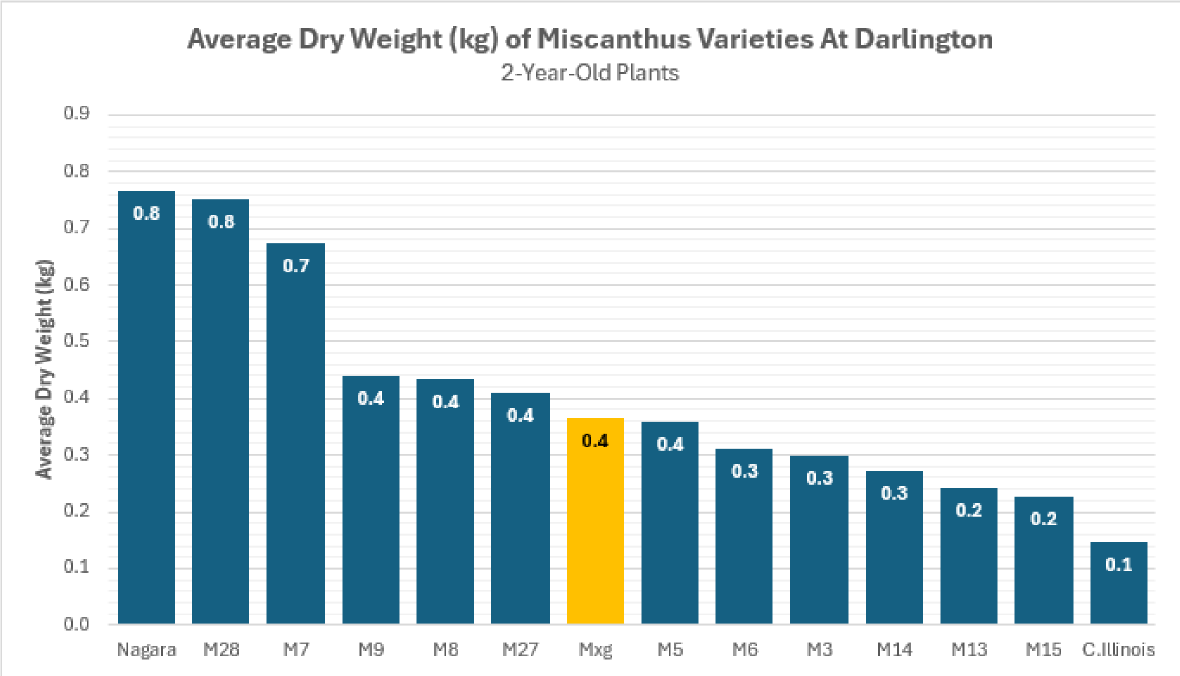

Figure 16: *Miscanthus dry weight plant yields, from specimen plots, Darlington harvest 2025.*

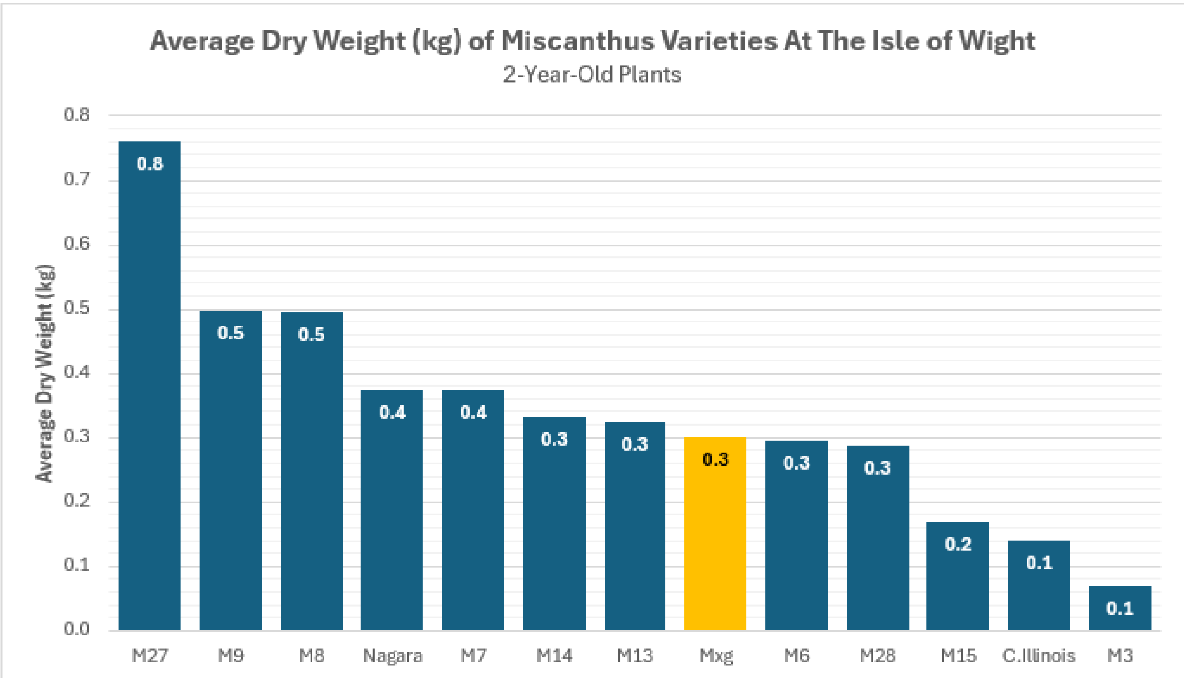

Figure 17: *Miscanthus dry weight plant yields, from specimen plots, Isle of Wight harvest 2025.*

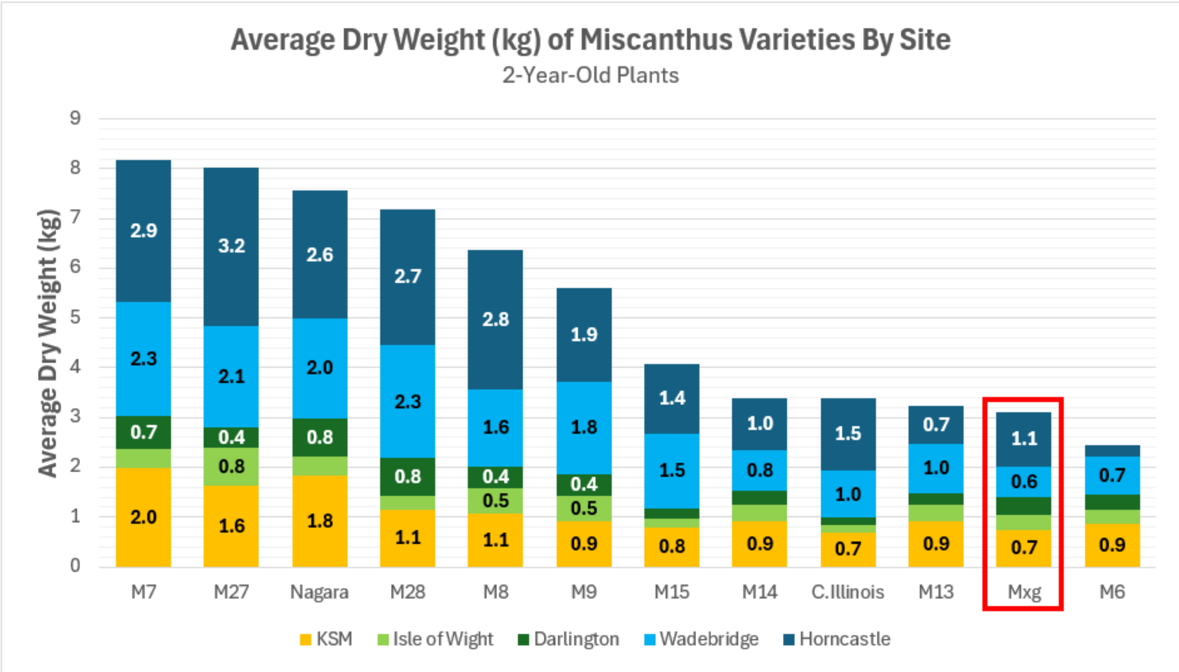

Figure 18: *Average dry weight of Miscanthus varieties at each of the five specimen trials.*

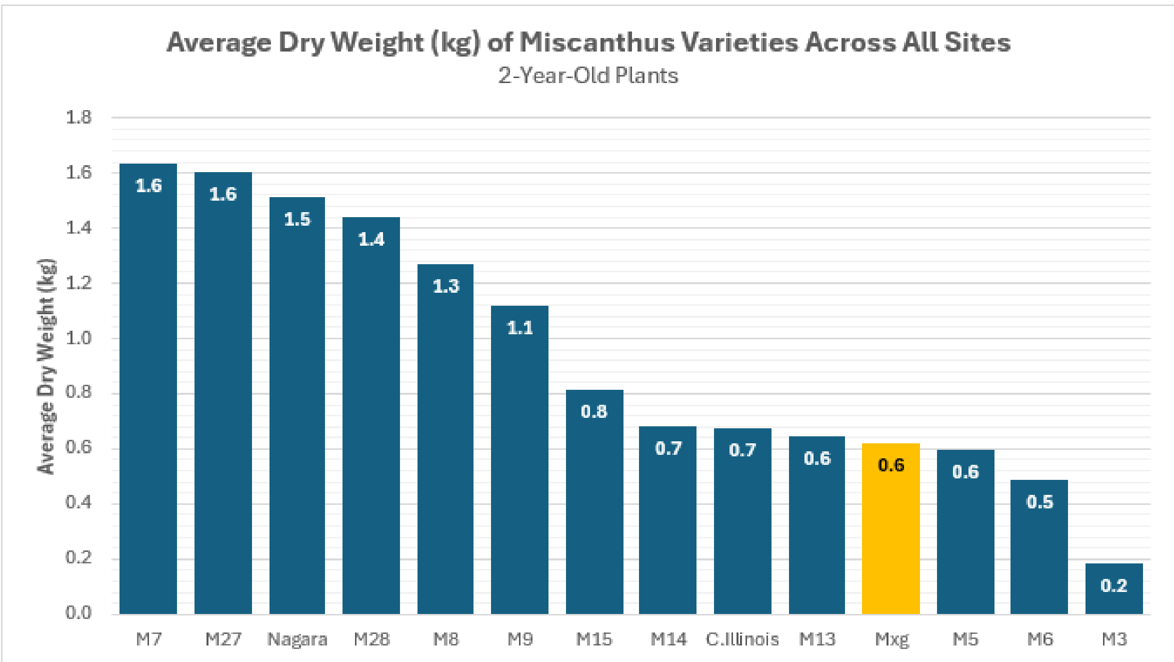

Figure 19: *Average dry weight, across five sites of each Miscanthus variety.*

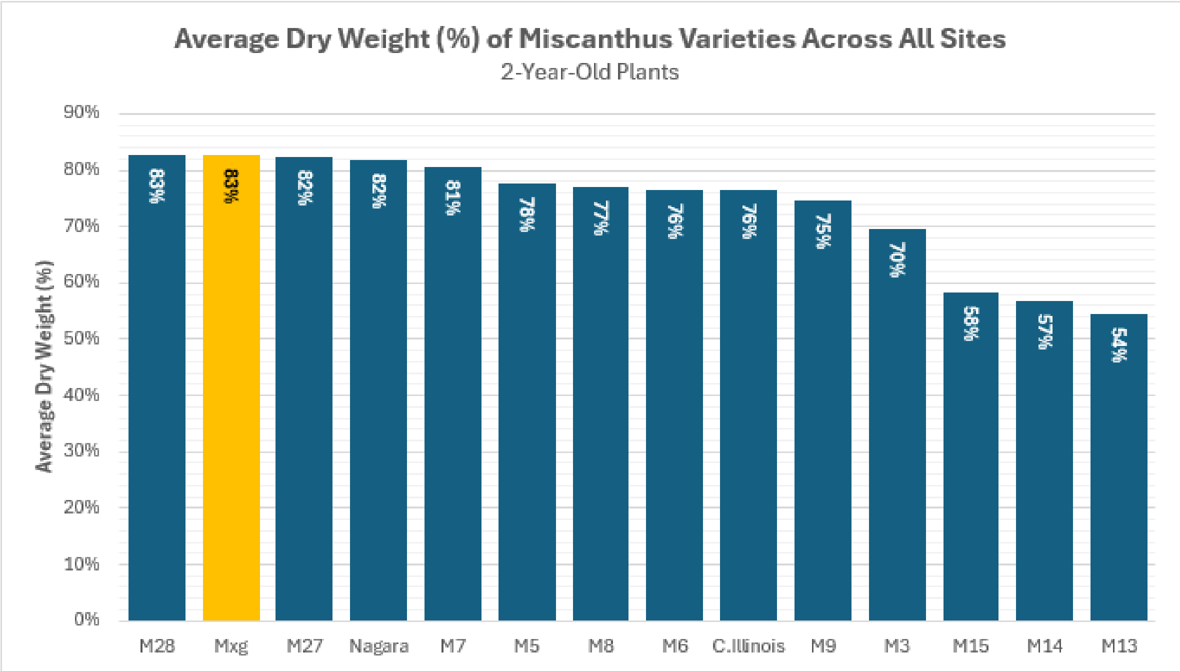

Figure 20: *Percentage dry weight of Miscanthus varieties across the five sites.*

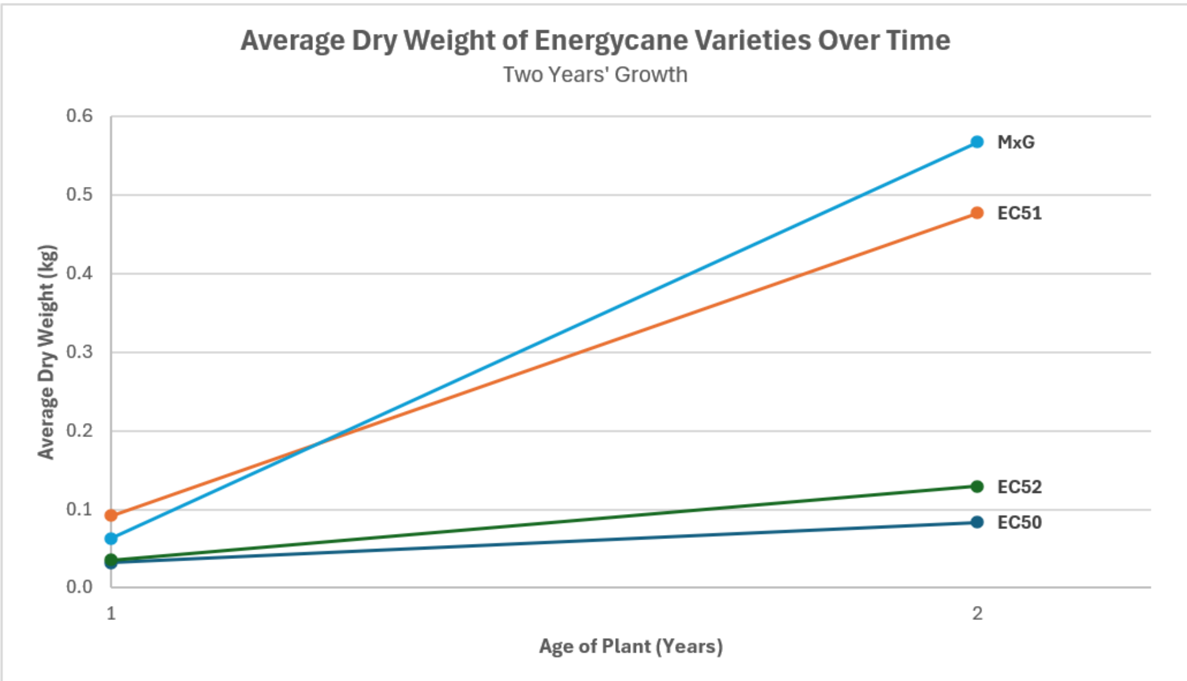

Figure 21: *Yield progression of the dry weights of Energy canes over two harvests.*

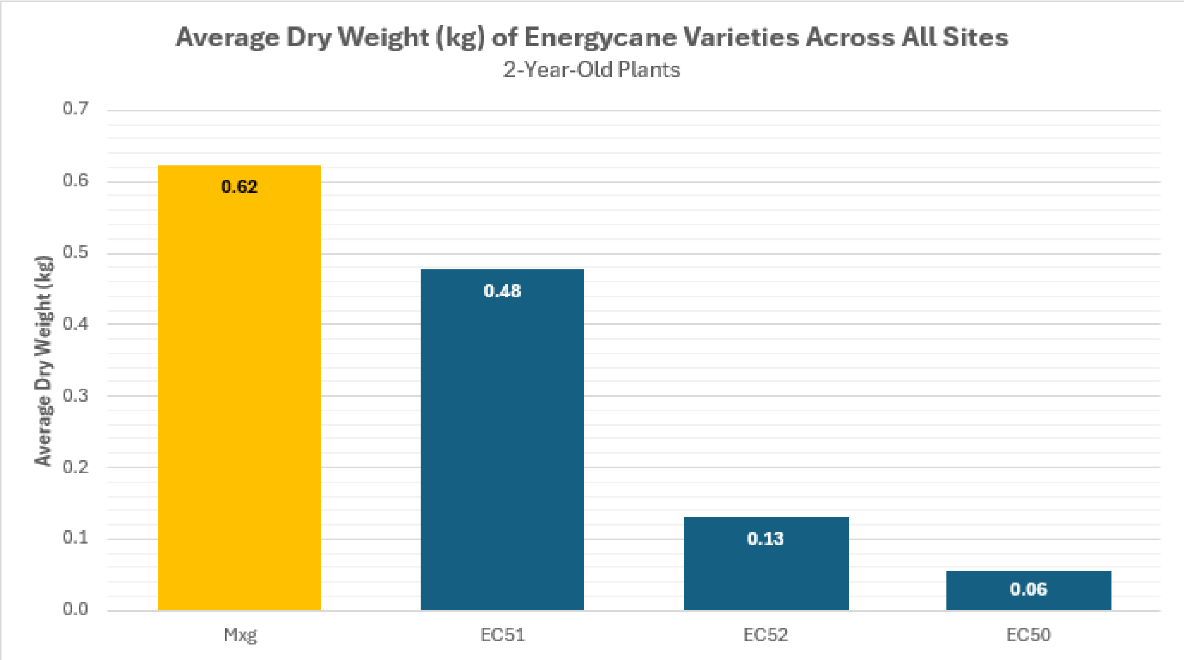

Figure 22: *Average dry weights of Energy canes after their second season of growth.*

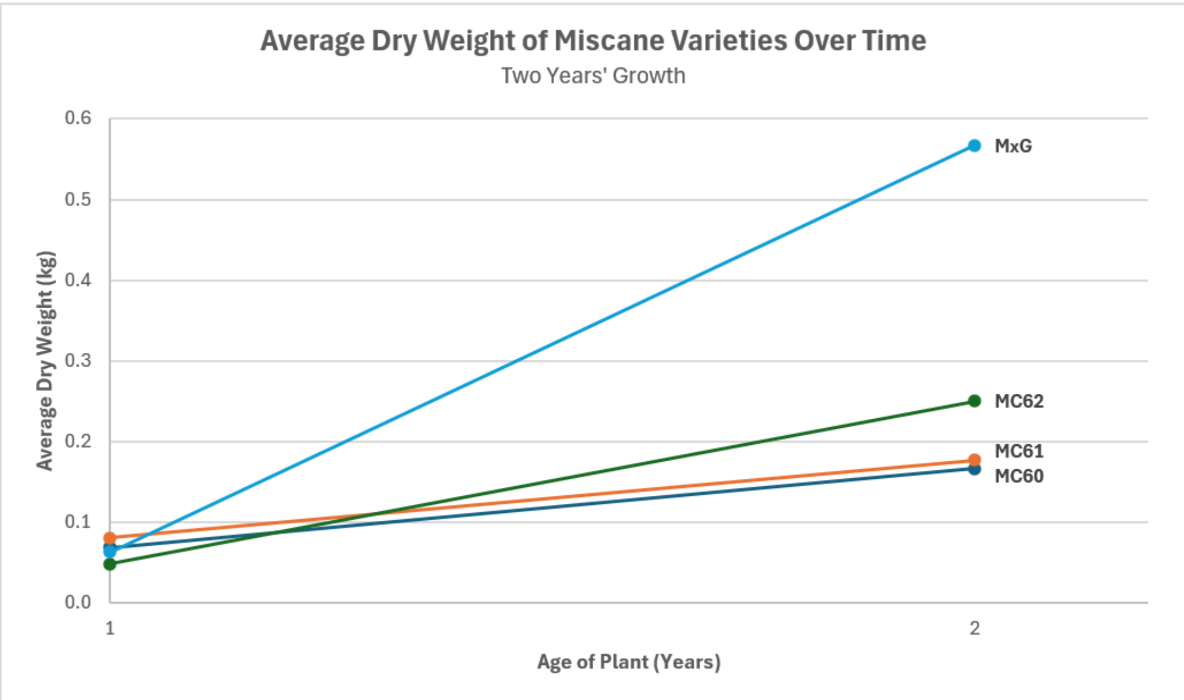

Figure 23: *Yield progression of the dry weights of Miscanes over two harvests.*

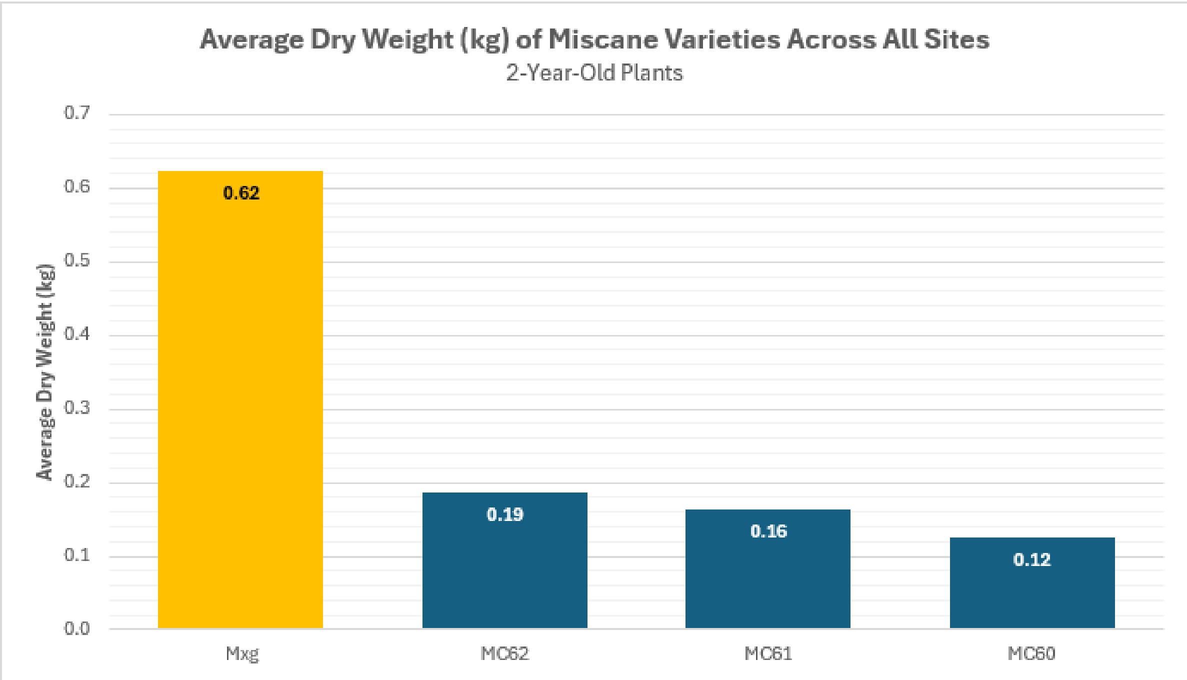

Figure 24: *Average dry weights of Miscanes after their second season of growth.*

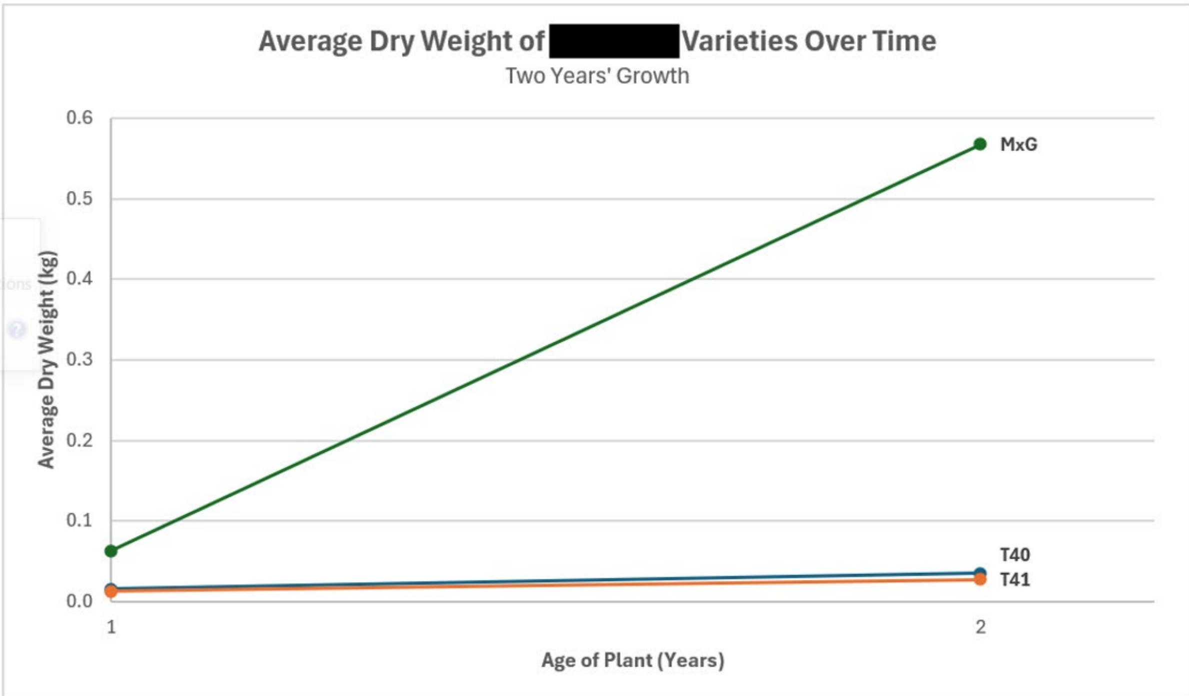

Figure 25*: Yield progression of the dry weights of* [redacted] *over two harvests.*

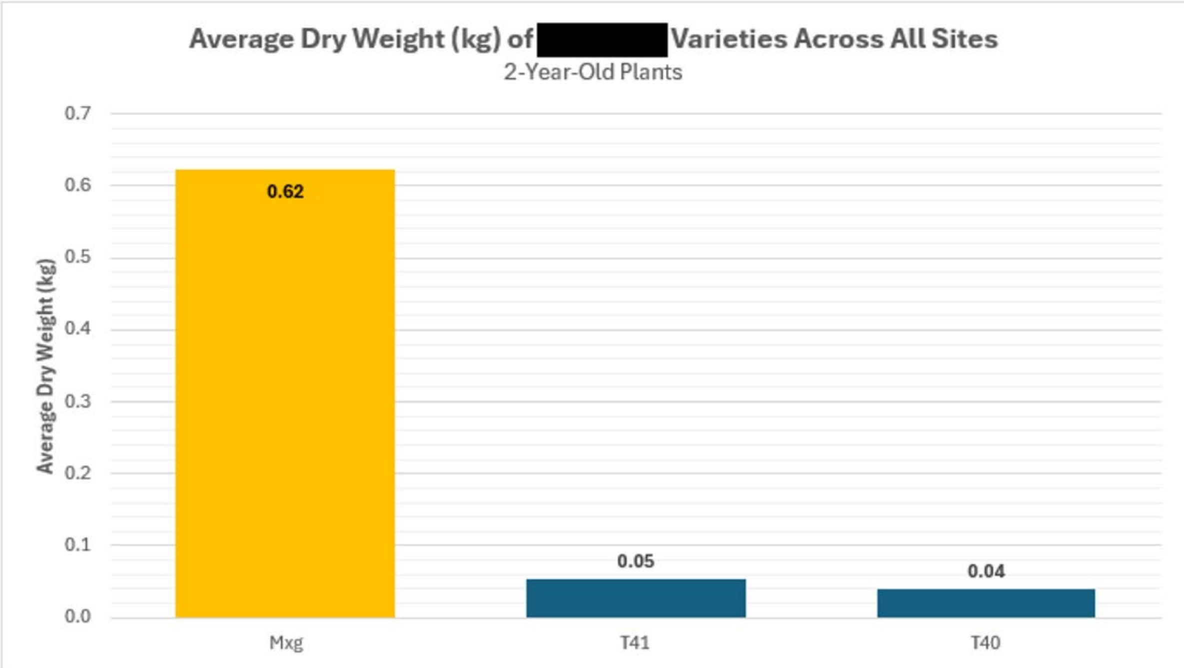

Figure 26: *Average dry weights of* [redacted] *after their second season of growth.*

[redacted]

Figure 27: [redacted] *growing in the UK, 23rd August 2023 (Redacted).*

[redacted]

Figure 28: *[redacted] growing in the UK, 23rd November 2023 (Redacted).*

Figure 29: *Image of Taunton trial of four [redacted] for AD analysis with maize plants growing at each end of the plot. 17th December 2024.*

Figure 30: *Taunton, 4th July 2023.*

Figure 31: *Taunton, December 2023.*

Figure 32: *Taunton, November 2024.*

Figure 33: *Taunton, late March 2025, prior to harvesting and early April after harvest.*

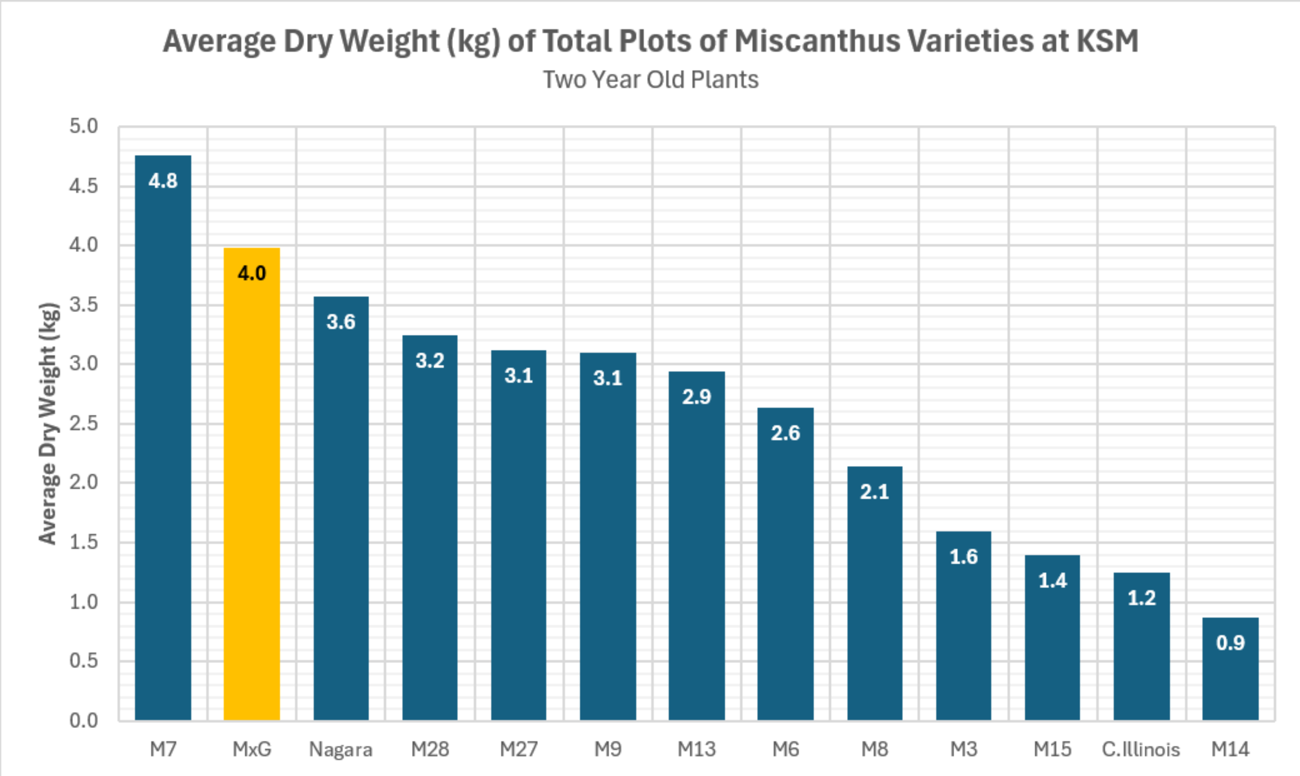

Figure 34: *Average dry weight (kg) of total plots of Miscanthus varieties at the Taunton site (coded KSM).*

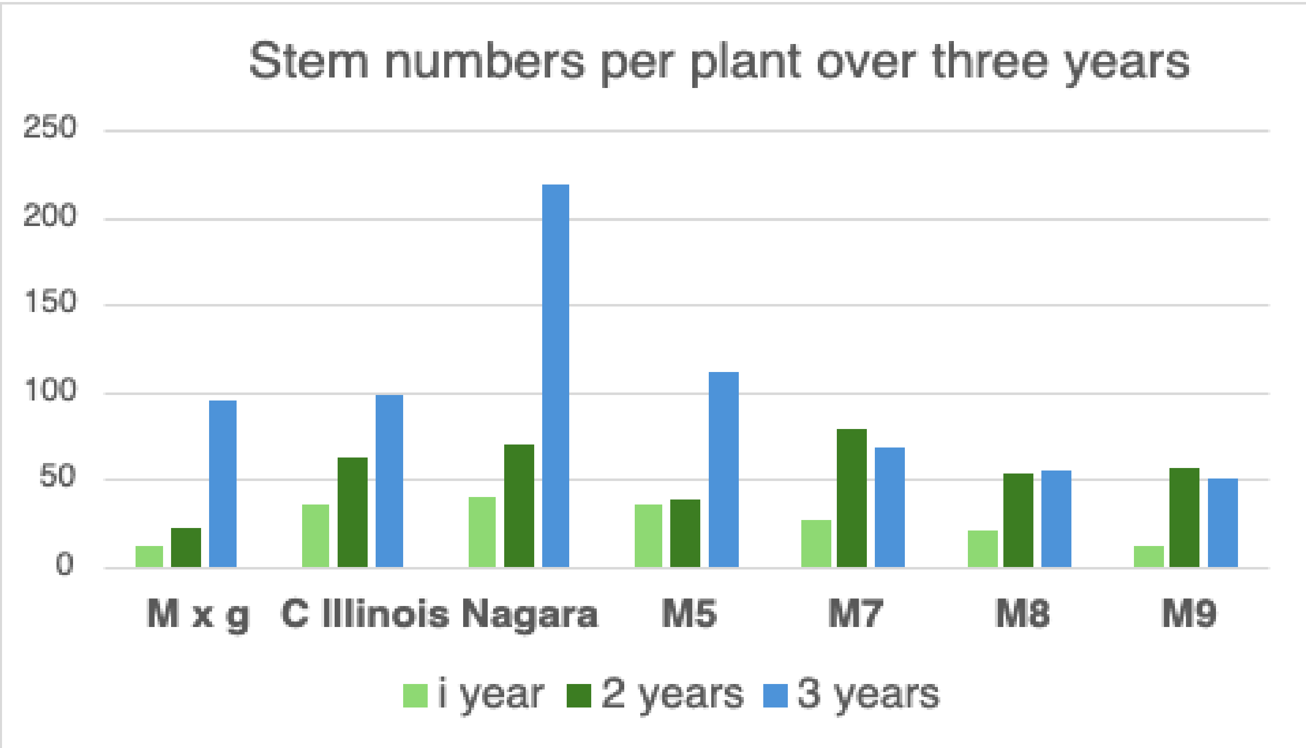

Figure 35: *Stem number per plant over three seasons, Miscanthus varieties.*

Figure 36: *Image of Taunton trial site (coded KSM), with specimen and variety plots, 15th August 2024*.

Figure 37: *Image of Taunton trial site (coded KSM), with specimen and variety plots, 17th December 2024.*

[redacted]

Figure 38: *Images of* [redacted] *growing in Spring 2025, showing greatly enhanced early growth compared to other years of testing.*

Annex 2 – Supporting information for WP 2, Propagation.



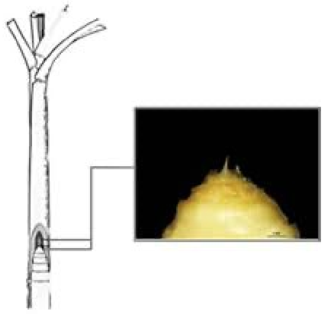

Figure 39: *Images of typical propagation material. Left: Miscanthus rhizome cuttings; Middle: Energy cane stem cuttings; Right: The apical meristem from a sugarcane plant used to start producing tissue culture plants.*



Figure 40: *Left: Image of automated whole-stem sugarcane planting in whole-stem; Middle: Planter to lay in rows; Right: Final stem material in the rows before being covered up.*


Figure 41: *Miscanthus rhizome planting operations. Left: Automatic rhizome planter, WH Loxton Ltd; Right: Manual planter (Miscanthus Nurseries Ltd).*

Figure 42: *CEEDS™ bio factory production, intensive horticultural production facility, low land footprint, and a final coated CEEDS™ capsule.*

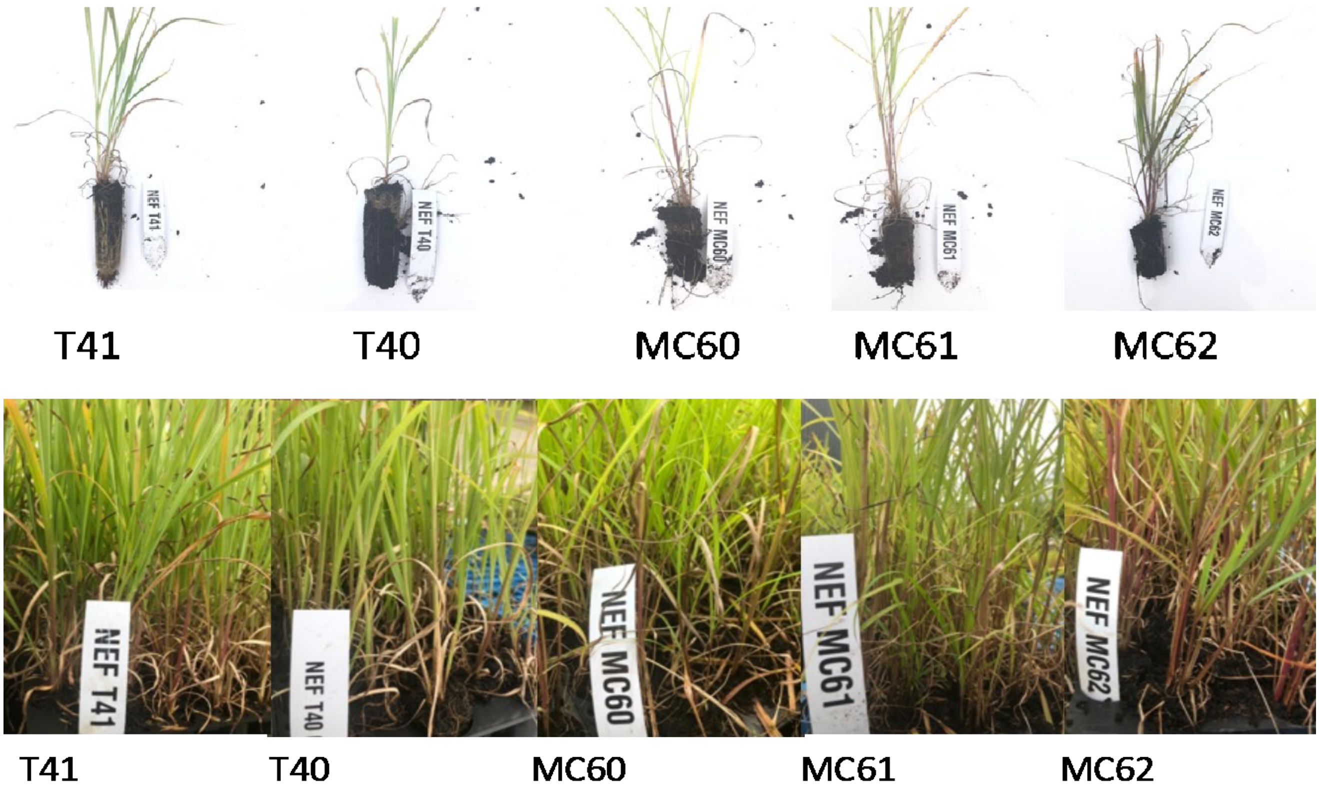

Figure 43: *Images of trial material established to evaluate mini rhizome and stem production capability with different species and varieties.*

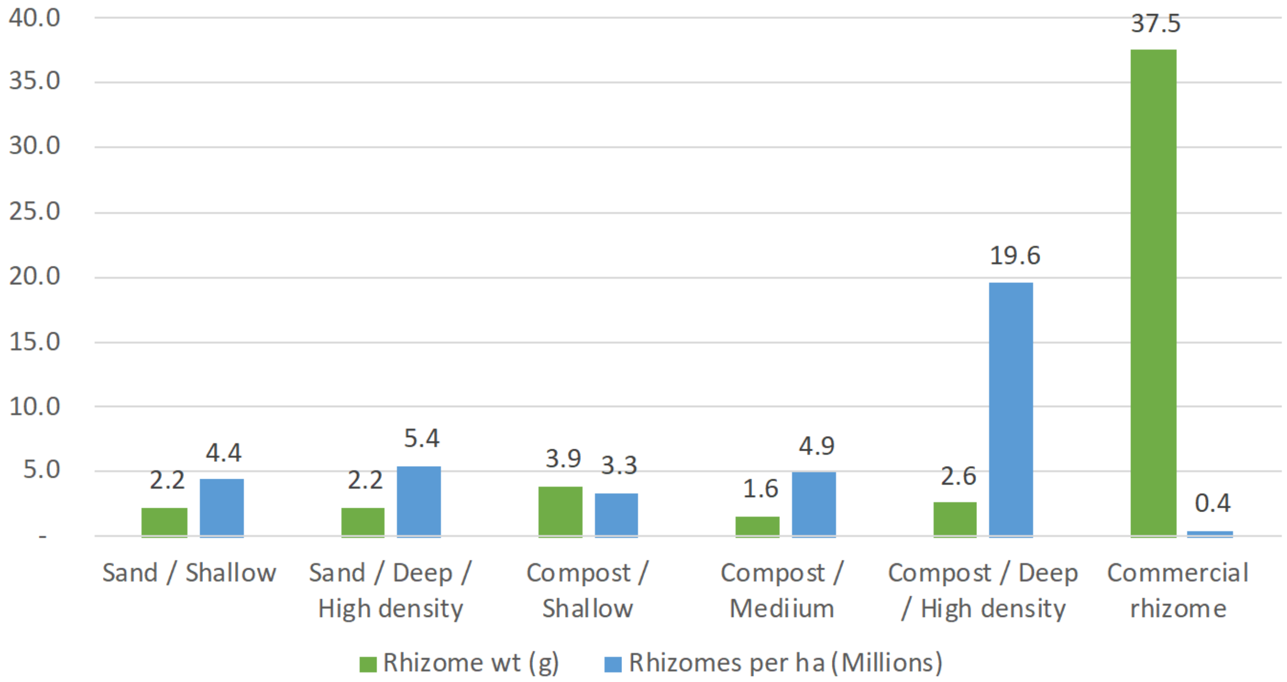

Figure 44: *Rhizome weight (g) and projected rhizomes yield per ha of growing space using different containers and growing substrates.*

[redacted]

Figure 45: [redacted]

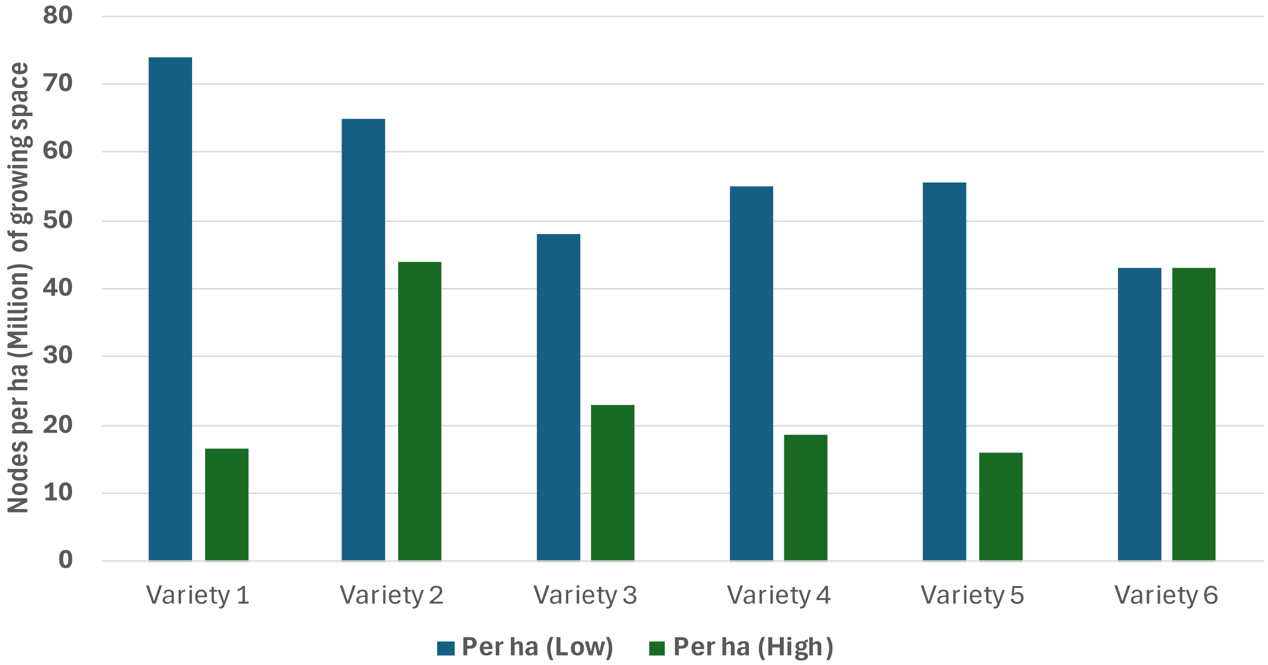

Figure 46: *Stem nodal production volumes,* [redacted].

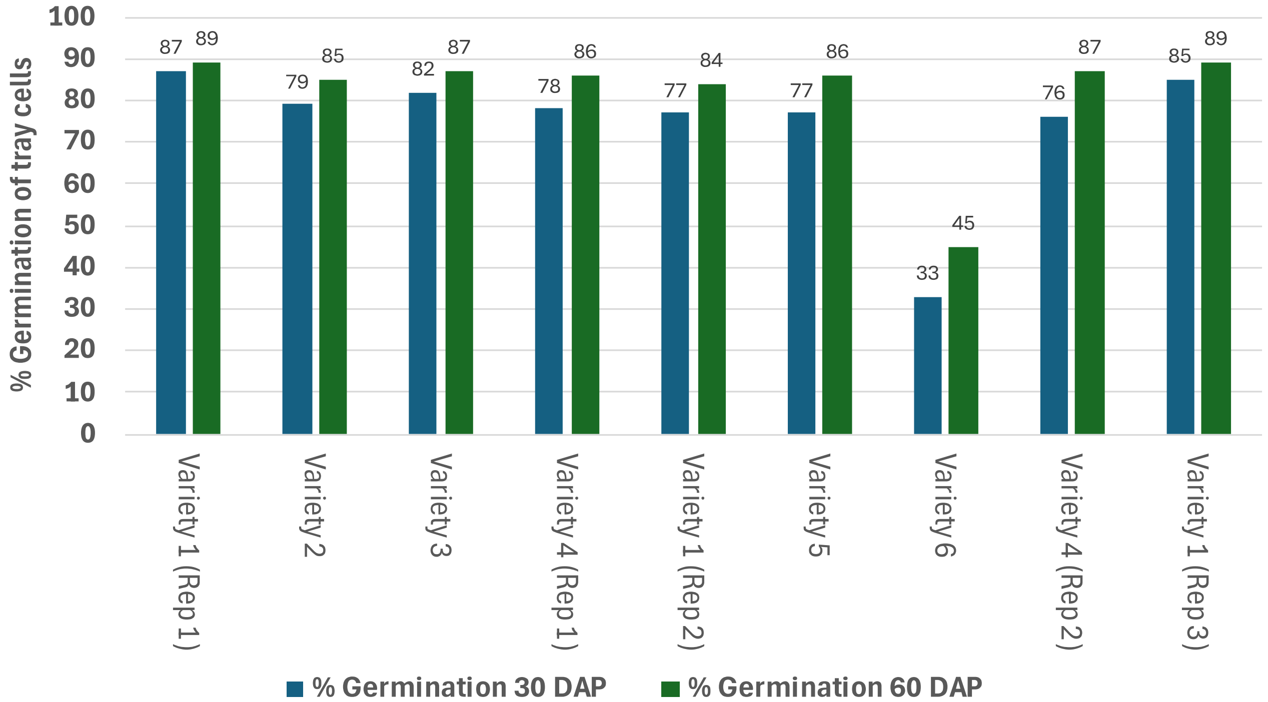

Figure 47: *Germination efficiency (%) of tray cells during hardening,* [redacted]*, after 30 and 60 Days After Planting (DAP).*

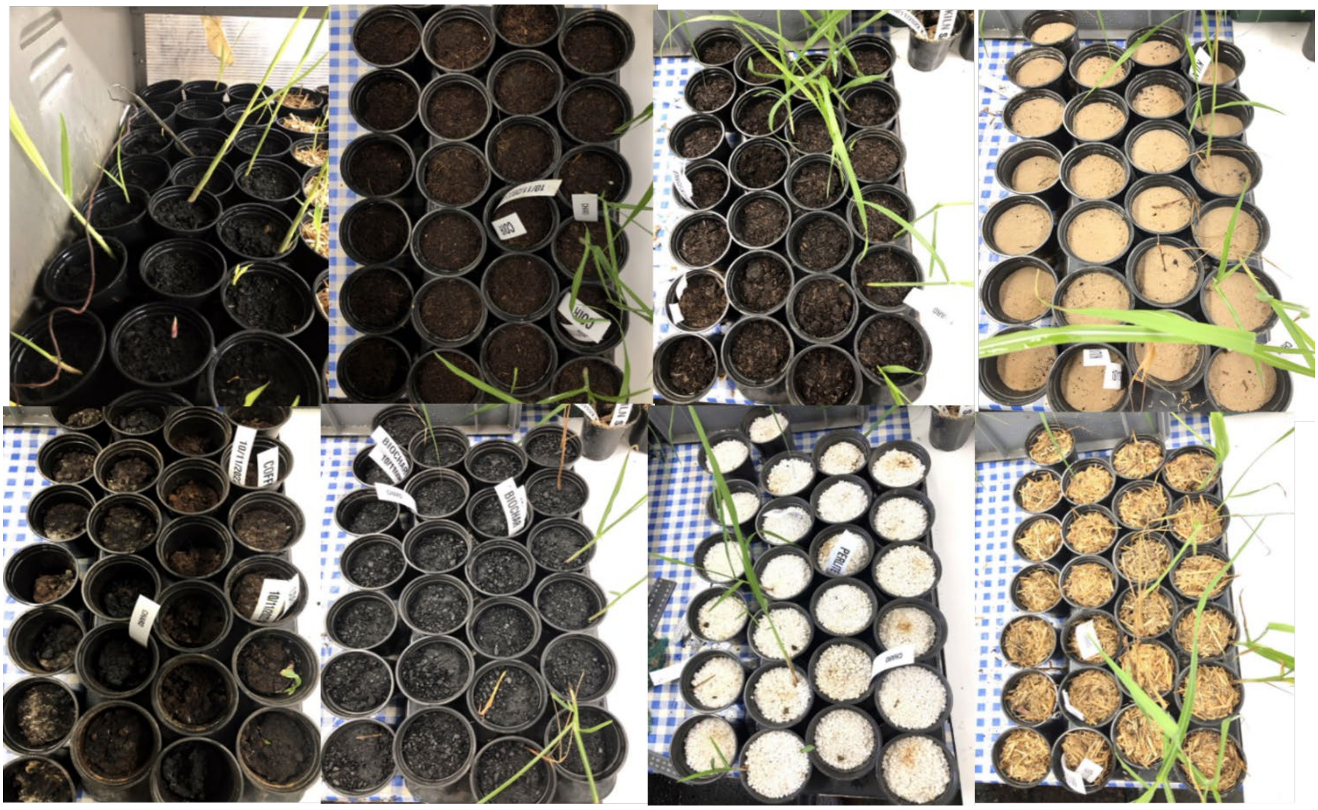

Figure 48: *Images of substrates tested for germination efficiency in hardening, ranging from conventional peat compost, AD digestate media, sand, Miscanthus chip, and perlite.*

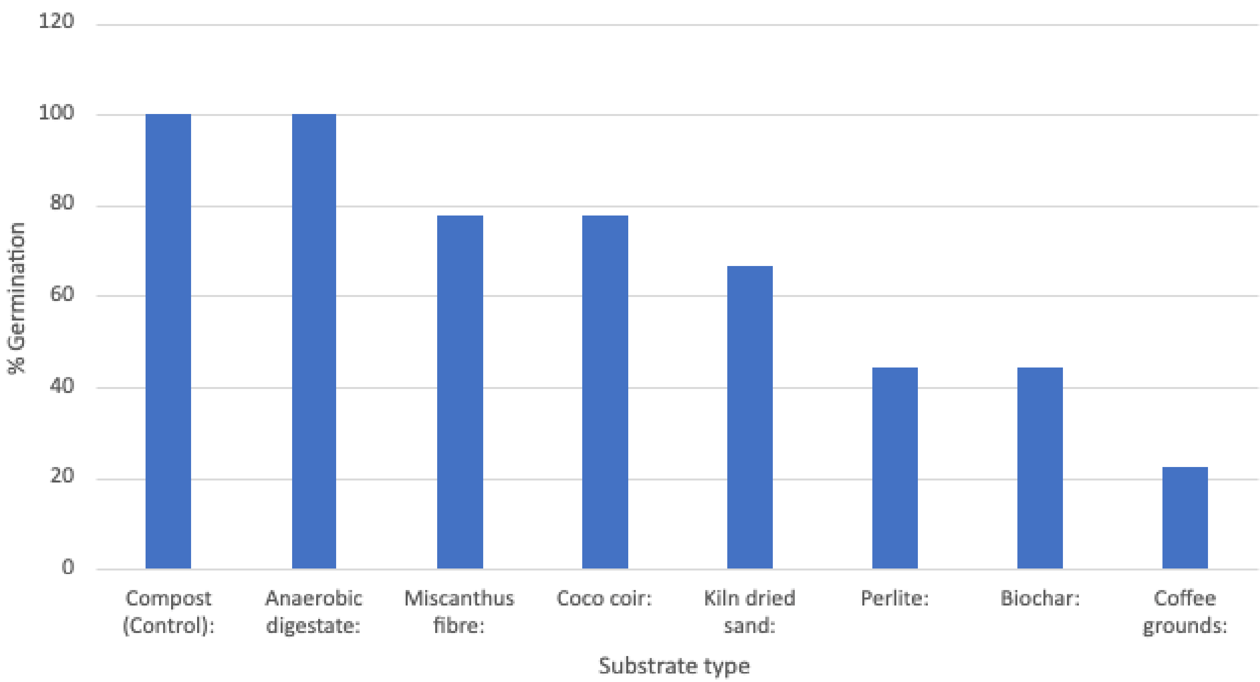

Figure 49: *Hardening protocols, testing of substrates, % germination in 77 cell trays after 40 days.*

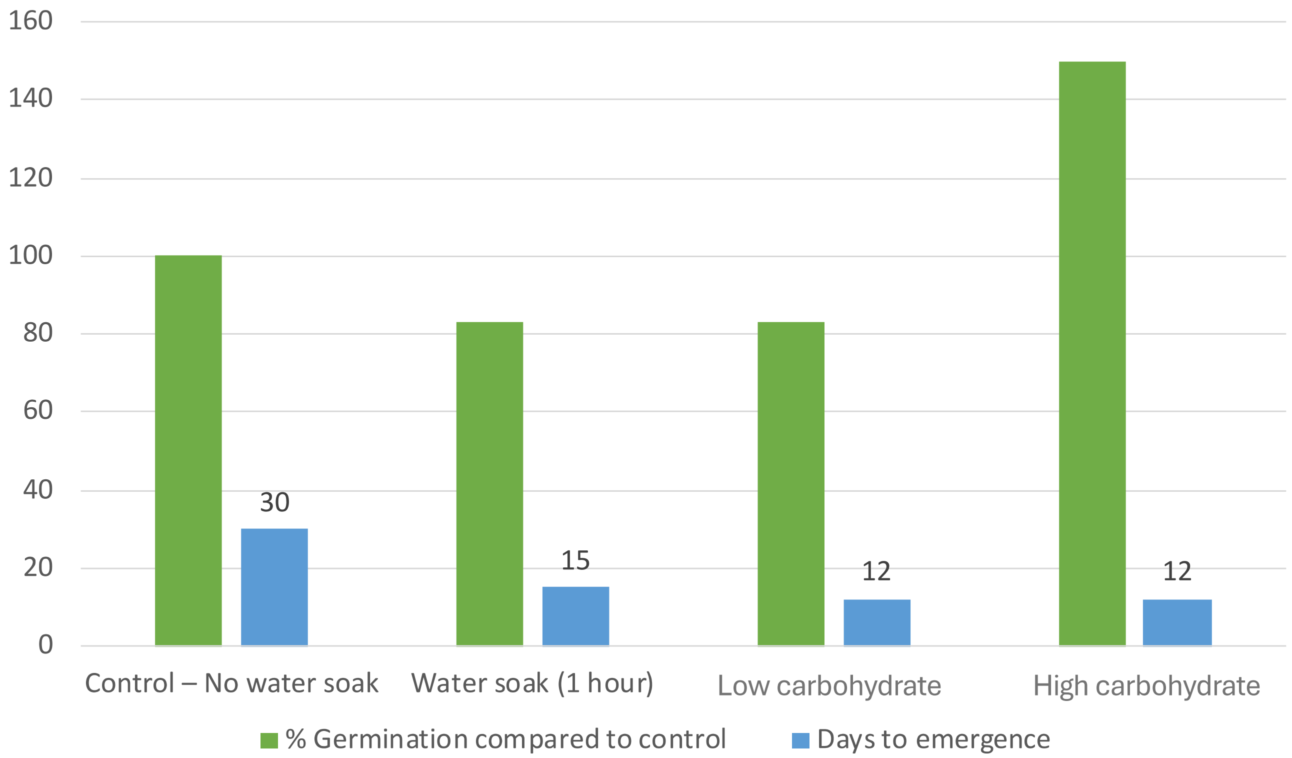

Figure 50: *Impact on germination speed and % (compared to control of no soak) with rhizome soaking in water and carbohydrate solutions.*

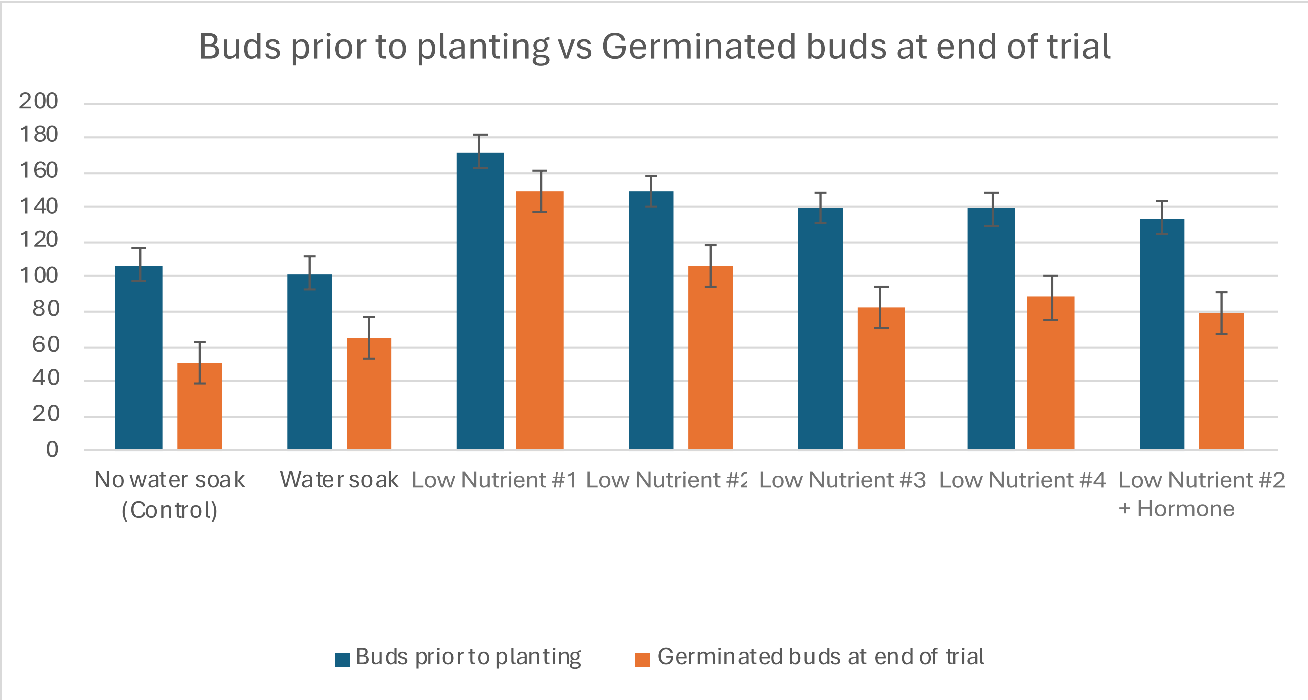

Figure 51: *Priming of Miscanthus* [redacted] *with nutrient soaks for 24 hours with different concentrations of single nutrients and one hormone* [redacted]*. Top: % rhizome bud germination at the start and end of the trial; Bottom: Average shoot growth (cm) of material after 42 days of growth.*

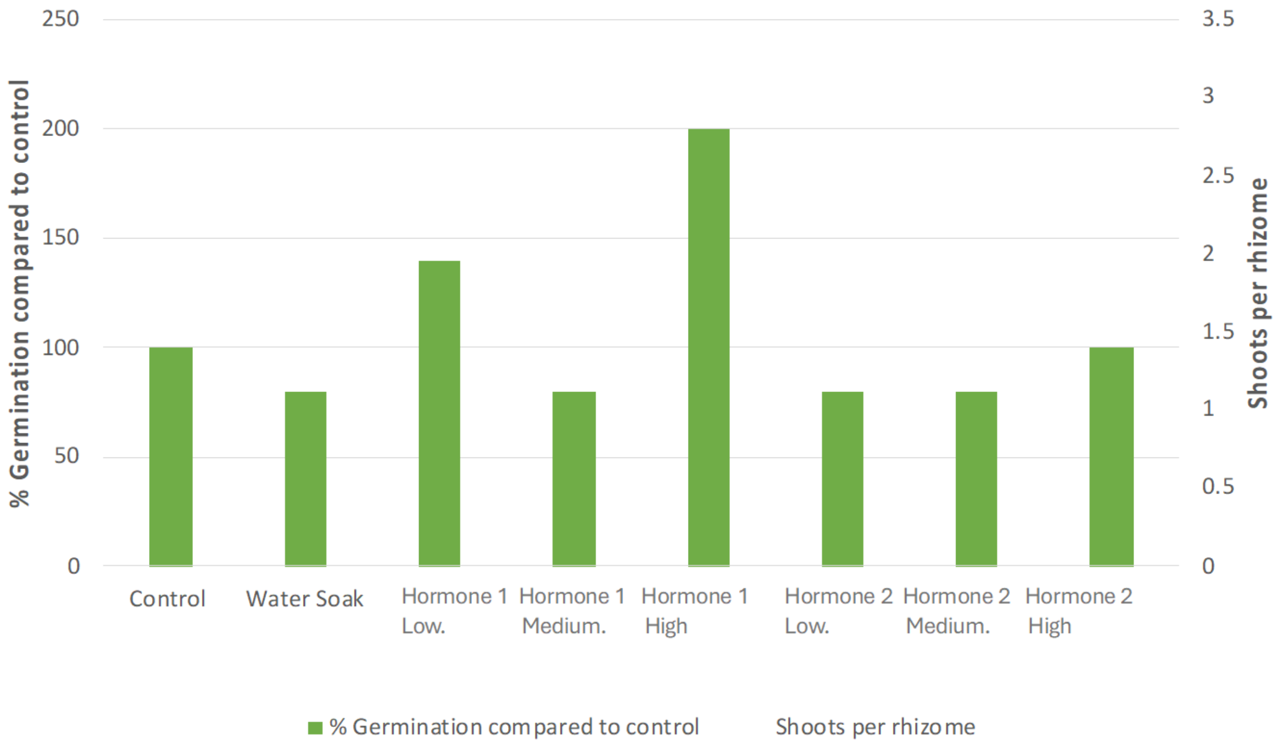

***Figure 52:** % germination and shoots per Miscanthus rhizome, with* [redacted] *treatments.*

Figure 53: [redacted] *and coated propagules with wax and biodegradable polymer prior to planting.*

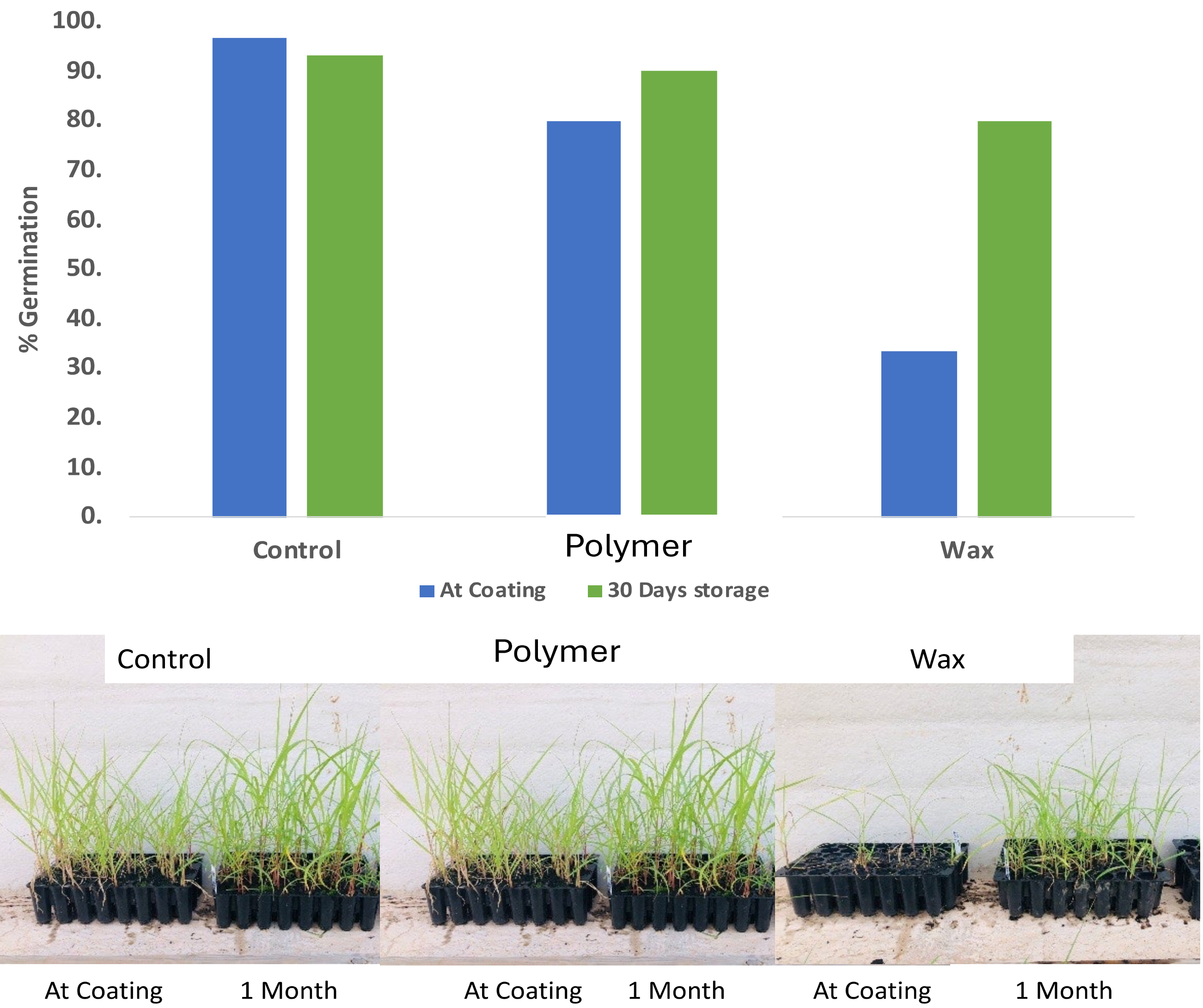

Figure 54: *Top: Percentage germination of Miscanthus CEEDS™ propagules coated in biodegradable polymer or wax, compared to an uncoated control after 40 days of growth, with the material assessed for germination immediately after coating, and with 30 days refrigerated storage at 3°C; Bottom: Images of material after germination testing with the different treatments.*

Figure 55: *Images of testing CEEDS™ movement and pick up inside existing potato cup planter units, as well as the existing potato planter used for these trials.*

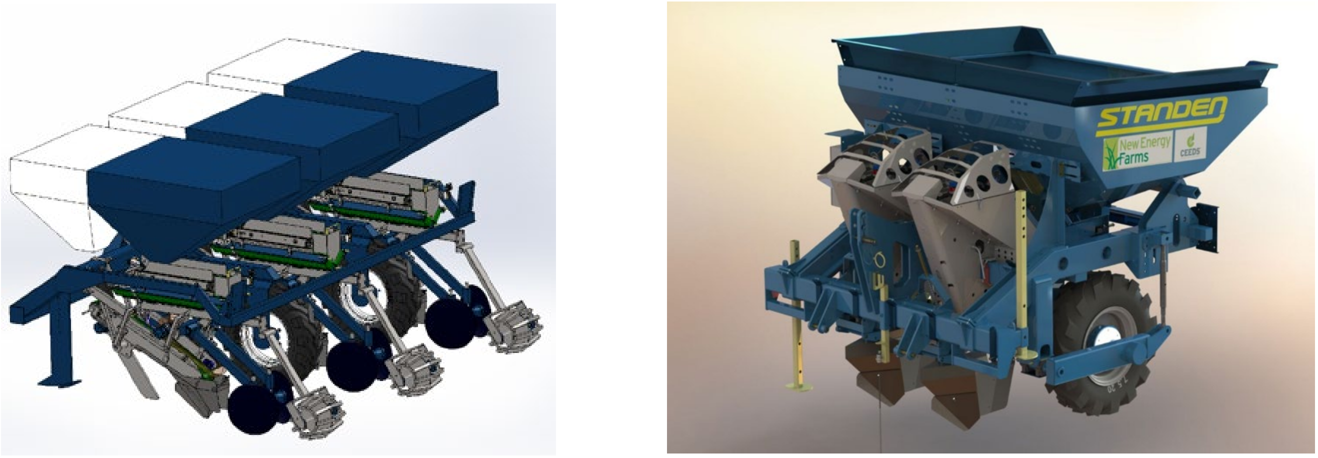

Figure 56: *Initial CAD design work and the final CAD designs for the CEEDS™ planter. This is a 2-row fully automatic planter.*

Figure 57: *Image of CEEDS™ field planter manufactured for UK energy crop planting.*

Figure 58: *Miscanthus CEEDS™ during early germination after planting on 22nd May.*

Figure 59: *Test planting of Miscanthus CEEDS™, material planted May 2022. Miscanthus CEEDS™ planting on the right, on the left, variety specimen plots.*

Figure 60: *Left: Plot plantings of CEEDS™; Middle: Image of a CEED™ germinating spring 2025; Right: Greenhouse germination testing.*

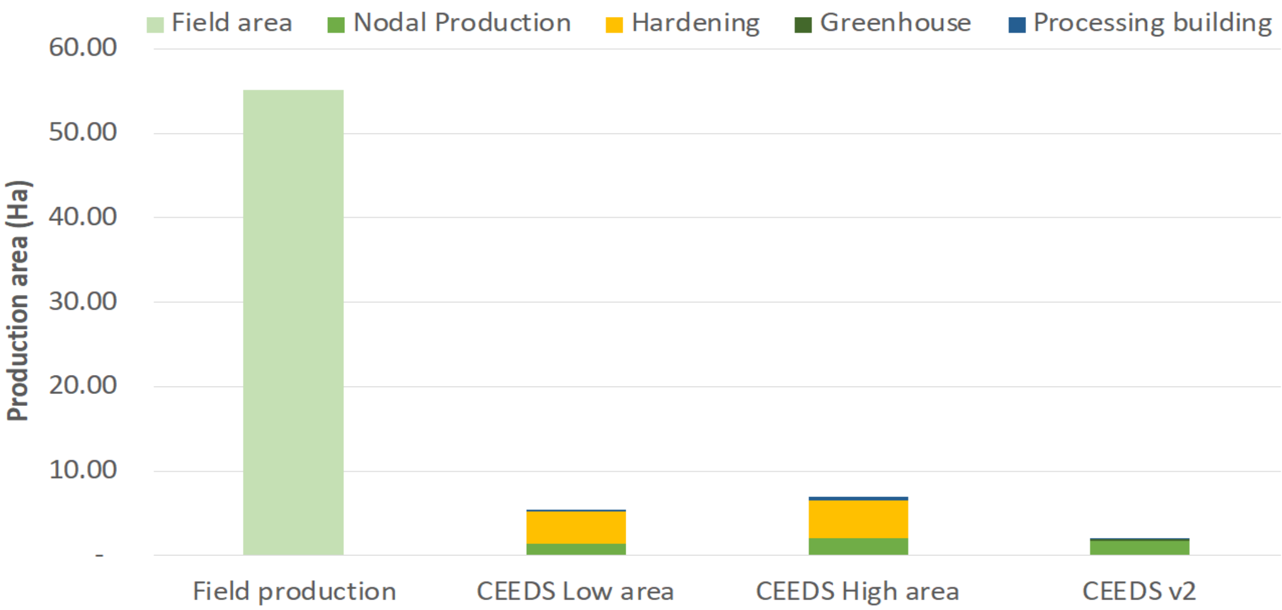

Figure 61: *Projected range of production area required for a 1,000-ha output per year CEEDS™ v2 bio-factory for rhizome propagated crops such as Miscanthus compared to conventional CEEDS™ prod. Production areas are broken down into different components.*

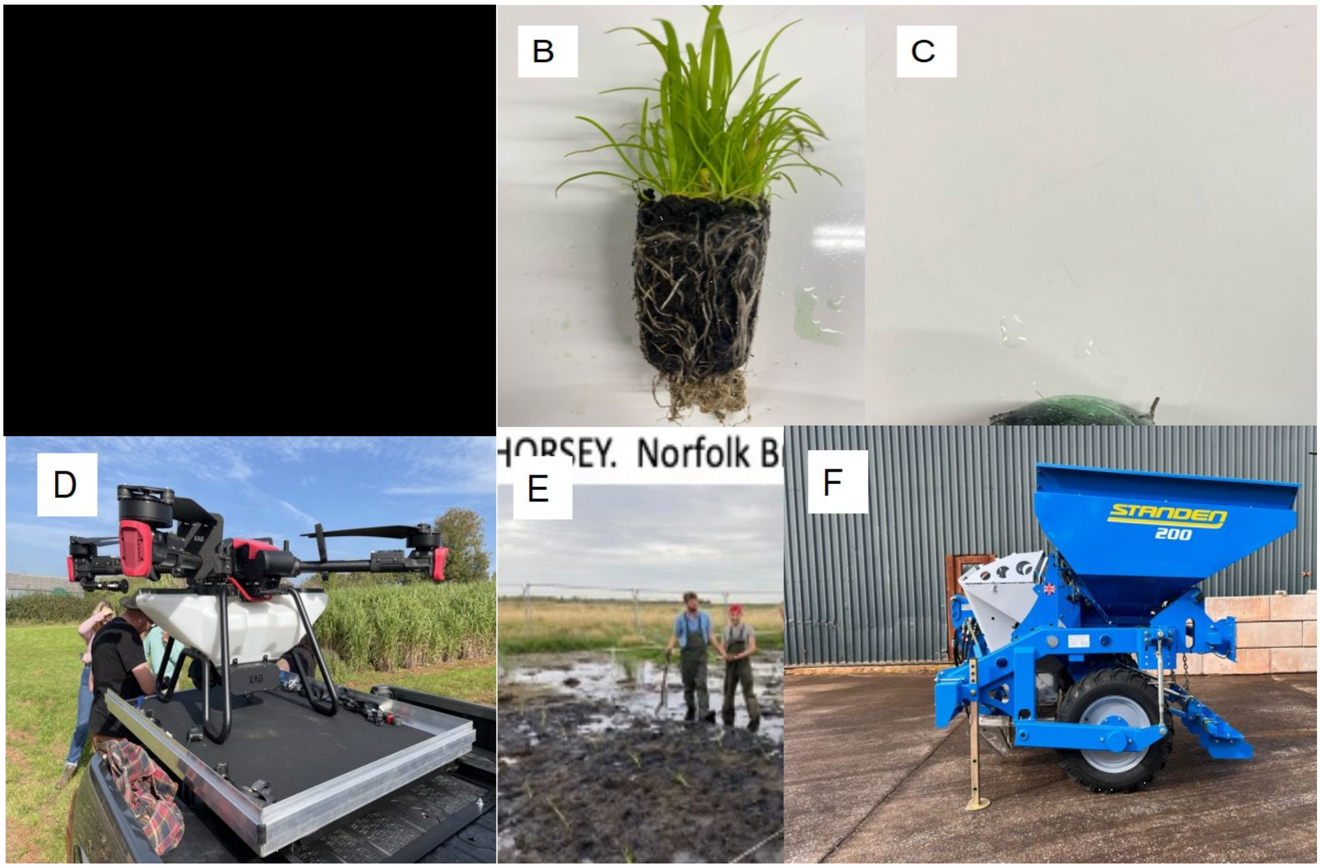

Figure 62: *Types of Typha propagules developed for commercial planting. (A) Drone planting balls; (B) Plug plants; (C) CEEDS™ with associated means of field planting; (D): Drone for balls; (E): Manual planting for plug plants; (F): Automated planter for CEEDS™.*

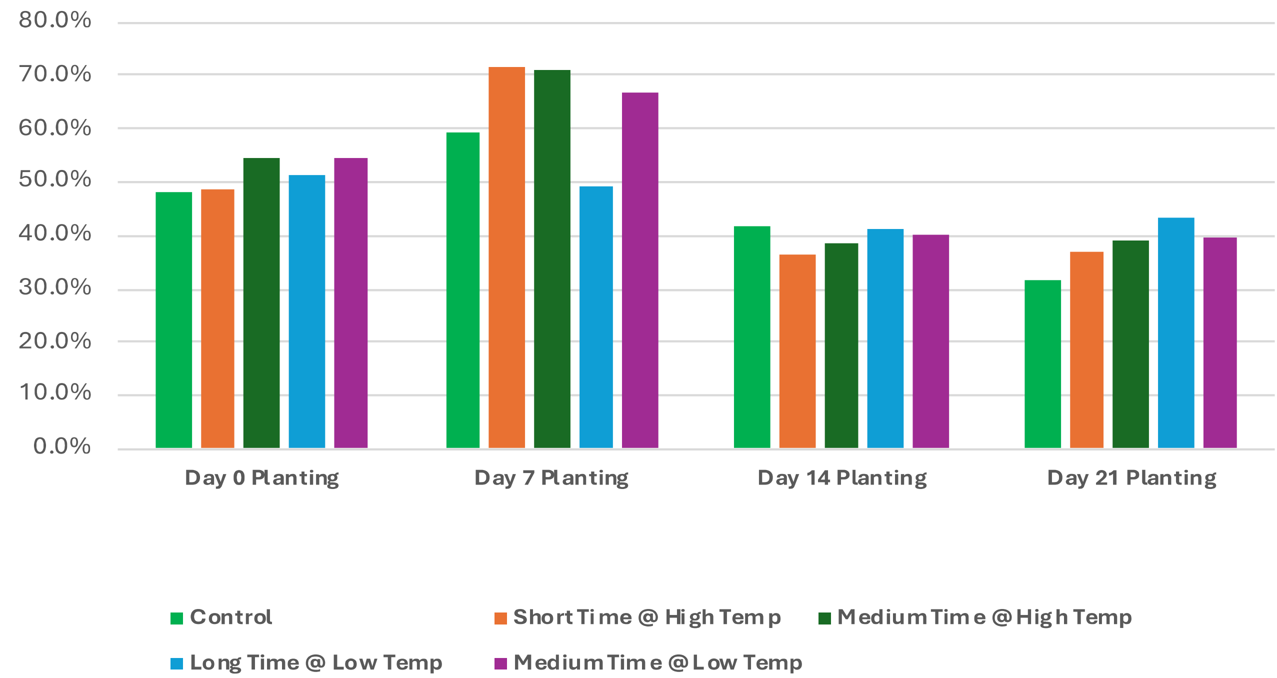

Figure 63: *% Miscanthus rhizome bud emergence after being primed and stored, material from Taunton site 2024.*

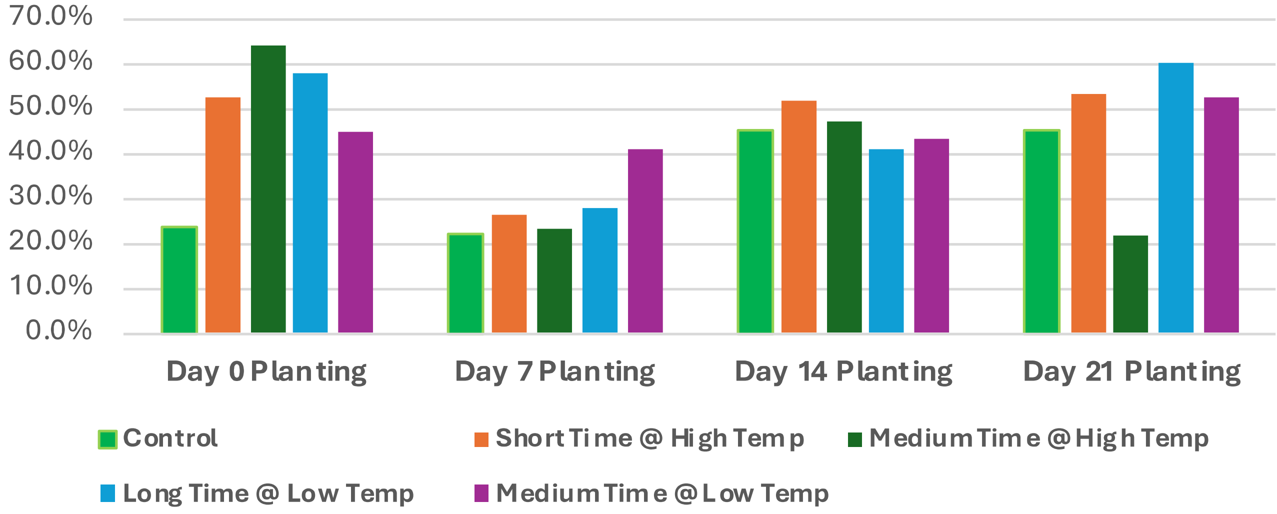

Figure 64: *Miscanthus rhizome % bud emergence after being stored and then primed, Lincolnshire site 2024.*

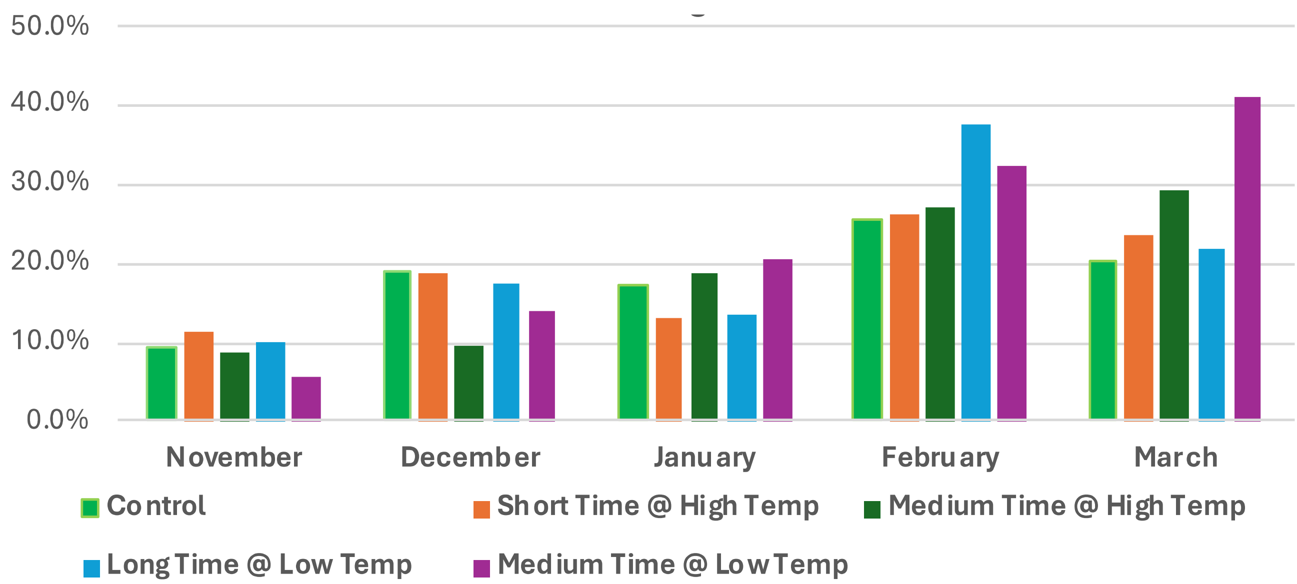

Figure 65: *Influence of lifting date on different priming treatments and % germination of early lifted Miscanthus rhizomes from November to March, material from Chard site, Somerset.*

Annex 3 – Supporting information for WP 3, Microbials.

3.1 Introduction

Capturing synergies between crops and endophytes (beneficial fungi and bacteria) is a rapidly developing technical area in world agriculture. Members of the Family *Poaceae* (Grasses) are responsive to microbials, a generic term favoured to describe these organisms. The market for *Rhizobium* (a genus of soil bacteria that fix nitrogen) and *Mycorrhiza* (*Arbuscular mycorrhiza* are the most common form of symbiotic association between a fungus and the roots of a vascular plant) is already considered to be just under $1 billion, worldwide. The fungal hyphae penetrate plant cells and develop branching morphological structures inside the cells, allowing a vast exchange surface between both organisms. The relationship that they can establish with the plant varies range from symbiotic to bordering on pathogenic. In New Zealand, endophytes are now commonly used in planting new grass pastures where once associated with the growing grass plants, they can reduce insect attacks.

During Phase One of the project, NEF completed a review of the international microbial market and specific research area opportunities within *Poaceae*. The conclusions were that the benefits of microbials include growth promotion, nitrogen fixation, disease control, insect control, stress (drought management) and tolerance of heavy metals. Microbial associations can be very plant-specific and climate-specific.

Initially, NEF explored the possibility of importing into the UK-specific microbes with known benefits of associating with *Poaceae* to test under UK conditions. However, this was not possible under current importation regulations**.**

NEF's studies were therefore focused on four research areas:

- Understand the microbial populations that currently exist in soils and on the root systems of commercial *Miscanthus* crops in the UK.
- Evaluate the extent to which associations can be developed between *Miscanthus* and the wide range of commercially available microbes in the UK market.
- Assess any longer-term effects of microbial associations in a controlled field trial situation.
- Explore the integration of microbials into CEEDS™ propagules.

NEF's collaborator in this work package was the University of Warwick (Professor Gary Bending, Department of Environmental Microbiology, School of Life Sciences). A New Zealand researcher, Nick Pyke, previously Director of The Foundation for Arable Research (FAR). It was initially intended to import a specific microbial formulation from Lincoln University, NZ, to Warwick University, UK, for trialling. Still, after extensive discussions, this was not allowed under current importation regulations.

3.2 Sourcing Potentially Suitable Commercial Microbial Formulations

In March 2023, four NEF staff members attended a Microbial Workshop hosted by Professor Bending at Warwick University. It proved to be a valuable meeting, stimulating excellent discussion about the direction and expansion of the studies, particularly the selection of likely candidates (fungi and bacteria) for greenhouse 'association' testing with young *Miscanthus* plants. Commercially available Microbial formulations were obtained from several UK companies. NEF also sourced Microbials from Europe, one of the two companies there was able to supply an endophyte which is reported from studies in New Zealand as having clear associations with *Miscanthus*. Previous NEF contacts with NZ researchers were critical in creating this Work Package. These formulations of fungi/bacteria, which are known to be capable of developing associations with plants (but not necessarily with *Poaceae),* were applied to plug plants of *Miscanthus* cv. Illinois in a fully replicated pot trial where their influence on plant growth was monitored. A total of seven microbial formulations were sourced for trials:

Commercial microbe formulation - Fungi and bacteria
Commercial Acidic Mycorrhizal formulation
Commercial Mycorrhizal formulation
Commercial application - fungal (dry)
Commercial application - fungal (liquid)
Commercial application - fungal (Foliar)
Commercial microbe formulation - Fungi and bacteria (hydroponics)

These microbial formulations were used in two glasshouse pot trials and one field trial.

3.3 Microbial Sampling of Commercial *Miscanthus* Crops

The sampling of commercial *Miscanthus* crops, which was conducted by NEF staff, took place over about three weeks in October 2022. Soil and rhizome root hair samples were obtained from 22 *Miscanthus* crops in England and delivered to Warwick University for microbial association analysis (Figure 66). The crops varied in their planting dates from 1998 to 2020. Soil samples were also obtained from each location for nutrient analysis.

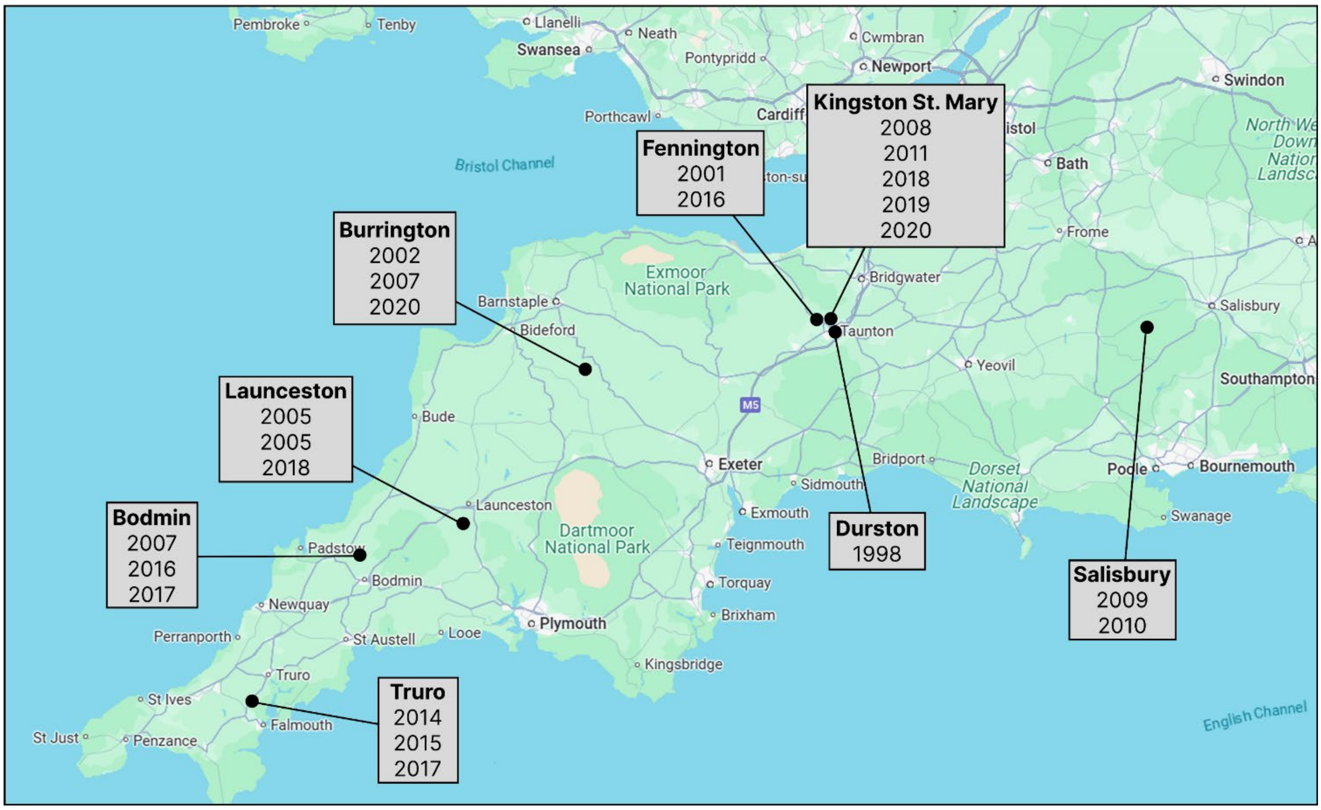

Figure 66: *Commercial Miscanthus crop locations sampled in 2022 for soil microbial associations, with crops between 2 and 24 years old (at the time of sampling).*

Table 10: *Location and age details of the commercial Miscanthus crop locations sampled in 2022 for soil microbial associations, with crops between 2-24 years old (at the time of sampling).*

Field Code	Grower	Location	Name	Hectares	Year Planted
[redacted]					2016
					2017
					2007
					2002
					2007
					2020
					2001
					2016
					1998
					2020
					2011
					2018
					2019
					2008
					2005
					2005
					2018
					2009
					2010
					2014
					2015
					2015
					2017

The sampling instructions received from Prof Gary Bending; University of Warwick (see below) need to be adhered to. Once removed from the field environment, microbial populations could deteriorate if incorrectly handled. 25 individual samples were removed from each of the 22 nominated crops, giving a total of 550 soil microbial samples and 550 root hair 'association' samples to be sent for microbial analysis to Warwick University.

Instructions for the collection of *Miscanthus* soil and root samples:

- Photograph the field/crop
- Walk into the crop at least 25 m from edges to an area of even establishment.
- Place cane in-ground and record GPS
- Using a trowel, remove approx. 150 g soil from 0-20 cm depth from 1m distance from the central point in N, S, E and W positions and place all four samples together in a single zip lock bag. Visually check samples containing roots. If they don't move the sampling closer to adjacent plants
- Seal the bag and label it with farm and sample position
- Place in a coolbox.
- Repeat this process at four further sampling positions at 25 m intervals along a line transect
- From each farm field, you will have five bags containing 600-800 g of soil
- Place in the fridge on return from sampling and transfer to Warwick for processing within three days

In addition to allowing soil and root hair samples to be taken from each crop, the collaborating growers provided management records of their crops and yields. We understand this is the most extensive microbial sampling programme ever undertaken in the UK on *Miscanthus*. Under the guidance of Professor Gary Bending, the dominant species of fungal and bacterial associations were identified through DNA extraction and sequencing. The overall results of the Warwick University analyses are presented in the attached Warwick University Report (Addendum 1) and highlighted at the end of this Report.

3.4 Test Microbial Formulations in Glasshouse Pot Trials

Seven microbial formulations entered glasshouse pot trials in 2023. Initially, eight commercial microbial products were identified as potentially suitable for trialling. Still, after further discussion with the production companies, one product was dropped from the trial because its application required specialised equipment and had significantly greater health and safety considerations. More details of the products used in the trial are presented in Table 11.

Table 11: *Details of products used in the microbial association trials.*

Treatment	Description	Type
T1	Complex mix of several bacterial and fungal components.	Powder
T2	Mycorrhizal fungi (acidic)	Granular
T3	Mycorrhizal fungi	Granular
T4	Trichoderma	Granular
T5	Trichoderma	Liquid
T7	Trichoderma	Foliar
T8	Fungi and Bacteria Hydroponic	Liquid

The *Trichoderma* Foliar treatment is marketed as a foliar application, but after discussion with the manufacturer, it was incorporated into growing media like the other treatments. One-year-old plug plants of *Miscanthus* cv. Illinois were planted on 13 February 2023 into field soil from the Taunton location that had been treated with one of the seven microbial products in a replicated pot trial conducted in a polytunnel. The consensus from the manufacturers was that associations could take up to six weeks to develop. This 'association period' is recommended for all incorporation studies and practices as it allows the microbes, and plant roots a significant time to incorporate. The soil used in the 'association period', and initial pot trial phase of the experiment was taken from the field where the plants would subsequently be planted in a field trial. The pot trial material was routinely irrigated, with periodic fertiliser treatments applied through the irrigation system. There were six replicates per treatment.

Plant heights were measured at the start of the trial and again after 42 and 98 days (Figure 67). The plants in the control treatment, with no addition of any microbial products, only increased in height by 1cm after 98 days. The most responsive treatment was 12 cm from T3 (mycorrhizal fungi). However, all the microbial associations produced overall increases in plant height compared to the control.

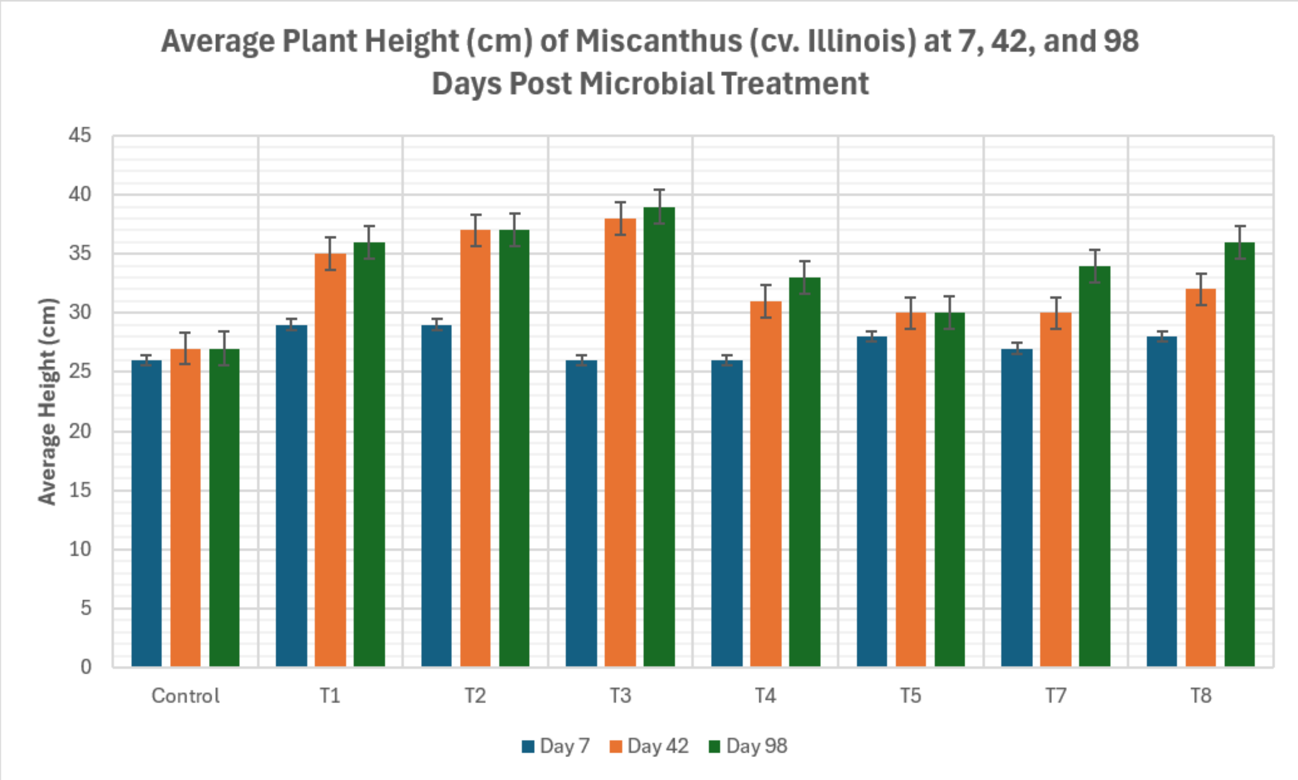

Figure 67: *Plant heights (cm) of the seven microbial treatments applied to Miscanthus plants (cv Illinois) compared to Untreated Control after 7, 42, and 98 days of growth.*

At the start of the trial, the individual plug plants were selected for uniform green leaf numbers on each plant. Leaf numbers were therefore counted again after 42 and 98 days. The results are presented below (Figure 68).

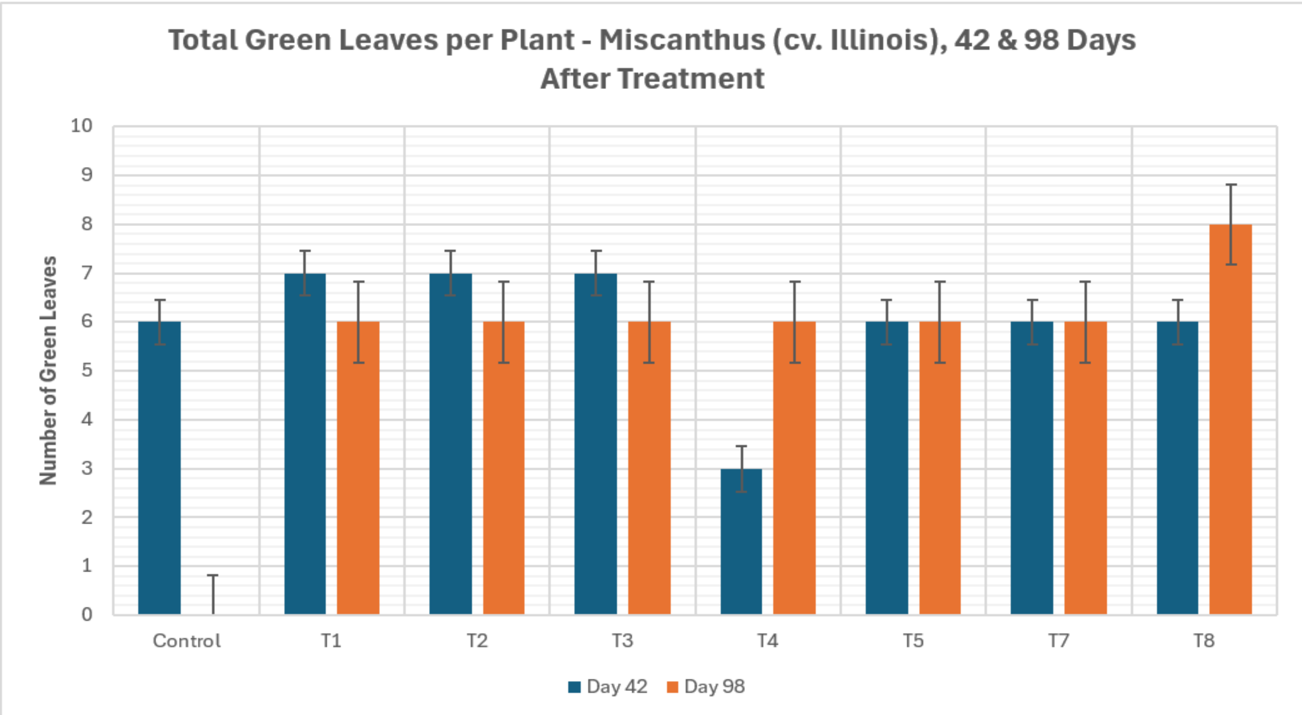

Figure 68: *Green leaf number per plant of the seven microbial treatments applied to Miscanthus plants (cv Illinois) compared to Untreated Control after 42 and 98 days of growth.*

Green leaf assessments revealed only minimal differences in leaf number between the microbial treatments after 98 days, but they all retained green leaves. In contrast, by day 98, the control plants had senesced, with no green leaves recorded, a very interesting observation.

This trial was due to be terminated once senescence occurred. However, the pot material was agreed to be transferred into the field to prolong the experiment and measure any further differentials between treatments. The individual plants from the Pot Trial (3.3) were transferred to a field trial at the Kingston St Mary, Taunton location on 24 May 2024.

3.5 Field Evaluation of Microbial Treatments

The field trial was located on an agricultural cropping field where lower and higher fertility soil areas were known to be present. The material from the eight treatments was split into two batches of plants, each half-planted in the low or high-fertility block.

At transfer and planting of the field trial, using the pot trial material, one further assessment of stem numbers and plant heights of all the material was undertaken (Figure 69).

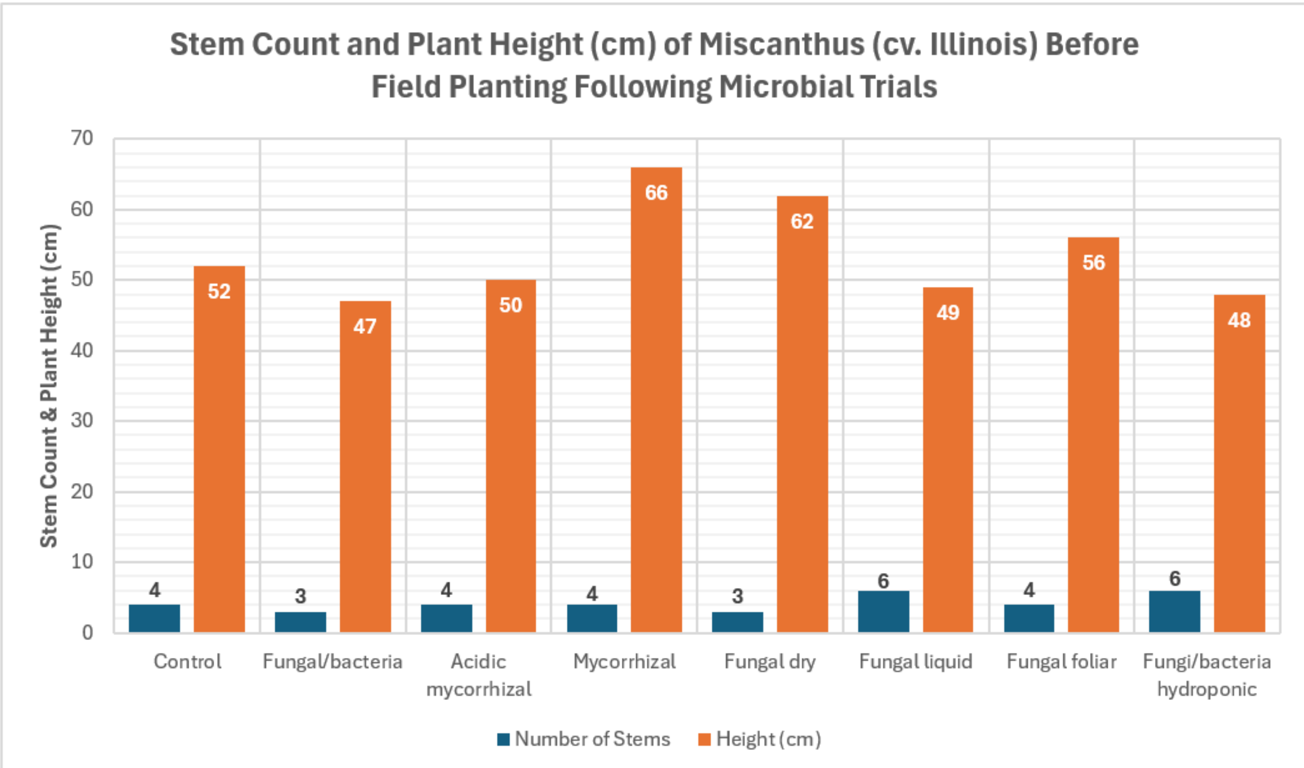

Figure 69: *Stem numbers and plant heights of the seven microbial treatments applied to Miscanthus plants (cv Illinois) compared to Untreated Control prior to field planting.*

The treatments of Trichoderma (T5 Liquid) and the Fungal/bacteria hydro (T8) liquid had the highest stem numbers at the point of transfer. Mycorrhiza (T3 granular) and Trichoderma (T4 granular) were the two tallest treatments.

At the end of May, a few days after the plant material had been transferred from the pot trial to the field location, the plants were assessed with a SPAD meter.

The soil plant analysis development (SPAD) chlorophyll meter is one of the most commonly used diagnostic tools for plants to determine the physiological activity of the plant. SPAD readings are calculated based on two transmission values: the transmission of red light at 650 nm, which is absorbed by chlorophyll and the transmission of infrared light at 940 nm, at which no chlorophyll absorption occurs. The measurements can monitor plant activity levels and indicate a plant's nitrogen status. Higher readings indicate more plant physiological activity.

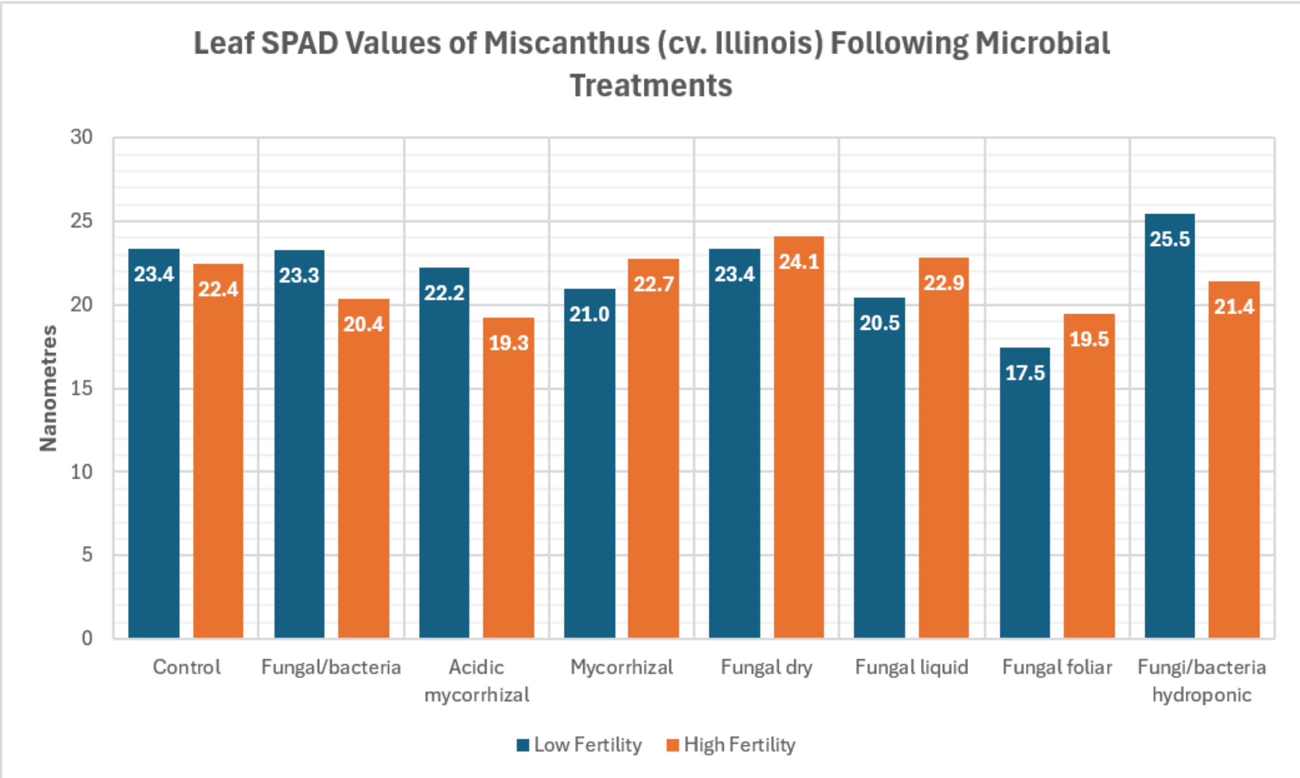

Figure 70: *Lead SPAD values of the seven microbial treatments applied to Miscanthus plants (cv Illinois) compared to Untreated Control prior to field planting.*

The SPAD values (Figure 70) in the low fertility treatments ranged from 17.5 to 25.5, and in the higher fertility plants, from 19.3 to 24.1. SPAD values were, therefore, quite similar across the range of treatments. The only treatment slightly lower in both fertilities was the fungal foliar treatment. The trial was harvested on 9 January 2025, 229 days after planting.

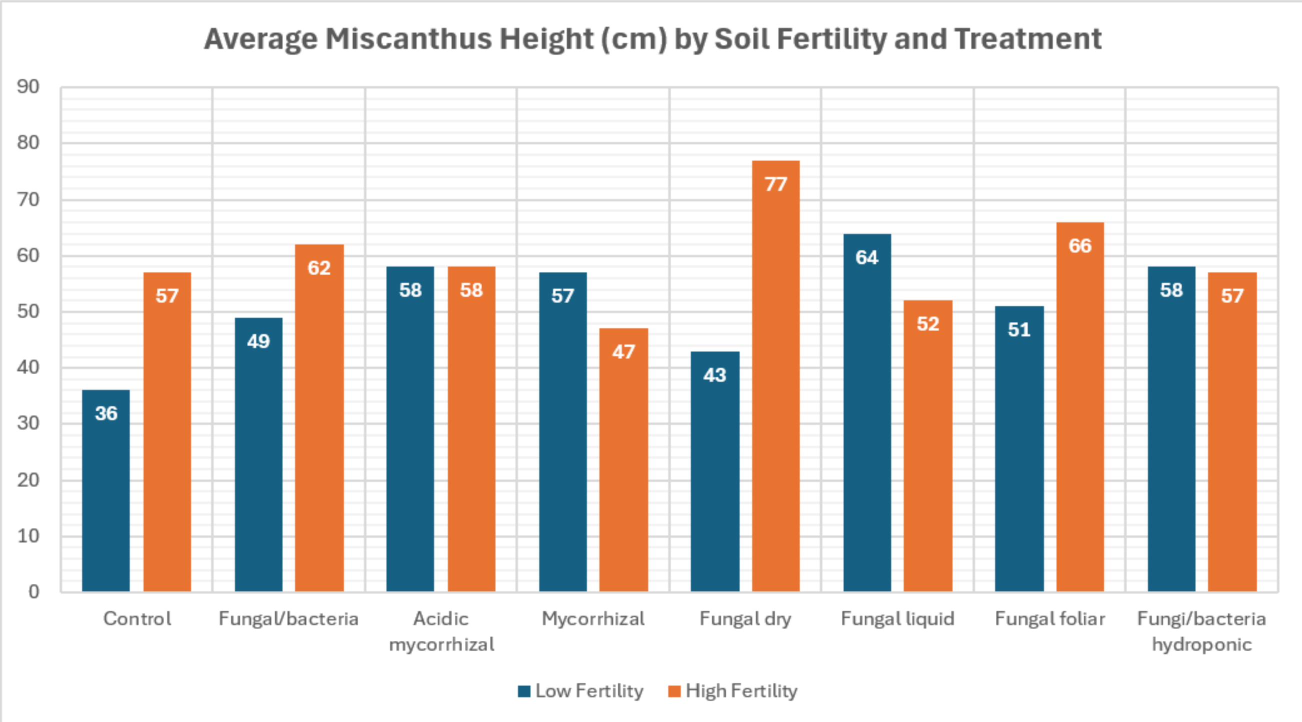

Figure 71: *Plant heights in the low and high fertility soils.*

All seven microbial treatments in the low fertility soil exhibited plant heights greater than those of the Control treatment (Figure 71), four of those responses being greater than a 50% height increase, mycorrhiza, mycorrhiza acidic, fungi/bacteria hydroponic and Trichoderma liquid. In the high fertility block, the most significant height response was from Trichoderma foliar, a 35% increase.

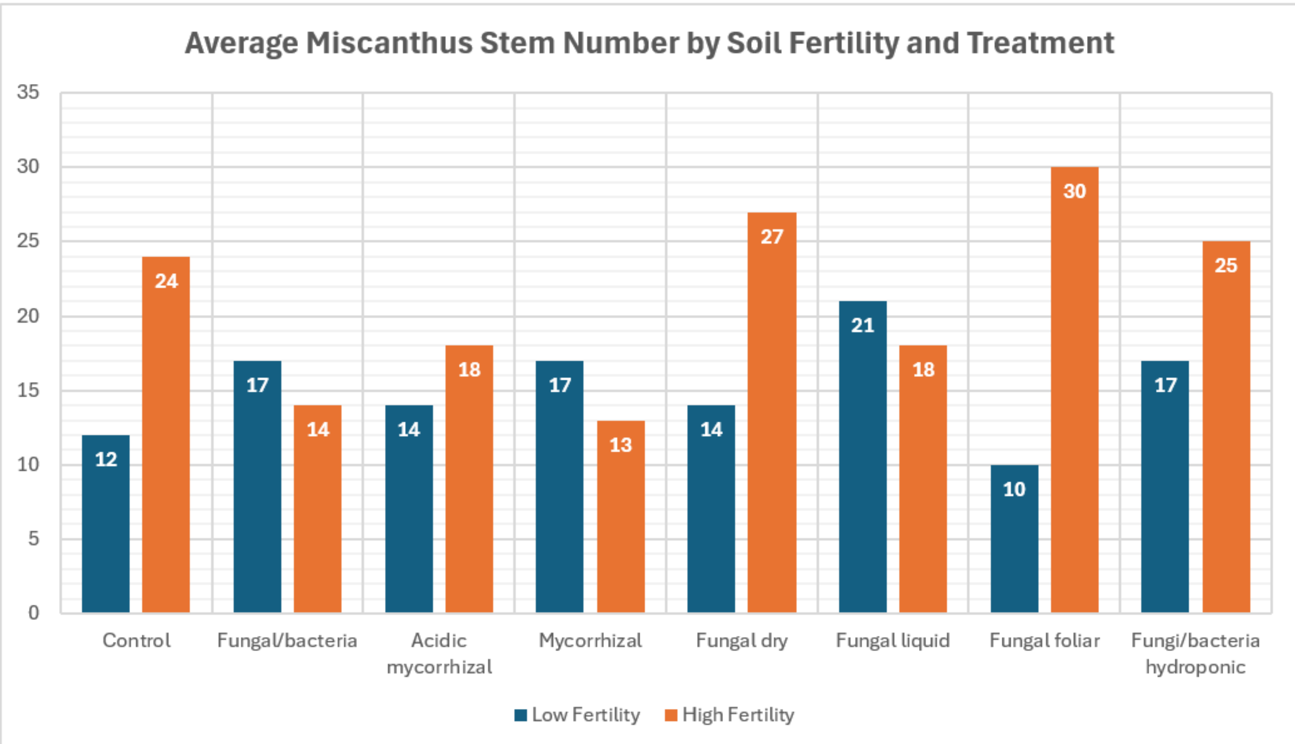

Figure 72: *Stem numbers in the low- and high-fertility soil.*

The highest increase in stem numbers in the low fertility soil was from Trichoderma liquid, 21 stems, compared to the control of 12 stems (Figure 72). In the high-fertility soil, the control increased average stem numbers to 24. The two highest treatments were Trichoderma foliar 30 stems and Trichoderma (granular) 27 stems.

The yield data, Table 12 and Figure 73 indicate that the average yield from the high fertility block of treatments was more than double that from the low fertility block. However, there appeared to be some interesting differences between the different treatments within the fertility blocks.

Table 12: *Average plant dry weights (g) from the eight treatments in the two fertility blocks.*

Code	Treatment	Low Fertility (g)	High Fertility (g)	% Change
Control	Control	9.2	55.3	+ 601
T1	Fungi and bacteria	19.4	35.0	+ 180
T2	Acidic Mycorrhizal	17.9	35.7	+ 199
T3	Mycorrhizal	37.0	19.6	- 47
T4	Trichoderma (gran)	14.1	95.4	+ 677
T5	Trichoderma (L)	35.4	40.8	+ 15
T7	Trichoderma (F)	8.8	63.5	+ 722
T8	Fungi and bacteria (hydro)	36.7	44.9	+ 13
Average		**22.3**	**48.8**	**+ 219**

The average plant dry weight increased from 22gm in the low fertility block to 49gm in the high fertility block. The lowest average plant treatment of 8.8gm was Trichoderma (foliar) in the low fertility block, the highest being 95.4 gm in the Trichoderma Liquid treatment.

The Fungi/bacteria treatment only produced a +13% yield improvement, and the Trichoderma liquid showed a 15% yield improvement when comparing the high fertility block with the low fertility block. The Control treatment had the third highest response to fertility, + 601% increase in yield, bettered only by the Trichoderma (granular treatment with a + 677% increase and the Trichoderma foliar with +722%.

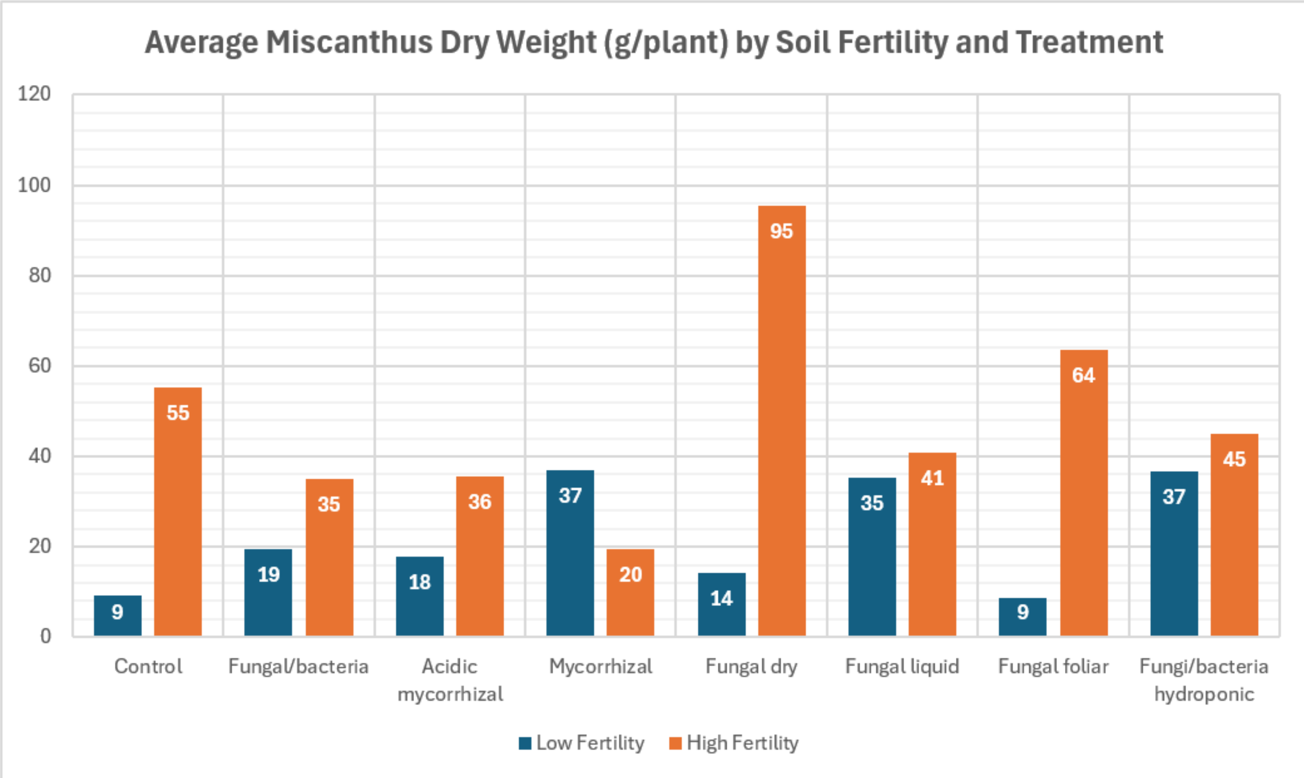

Figure 73: *Yields (dry weights) in the low- and high-fertility soils.*

Three treatments in the low fertility block produced very positive responses to microbial treatments: Mycorrhizal, Fungi/bacteria (hydroponic) and Trichoderma (Liquid). three other microbial treatment products, smaller yield increases. The Trichoderma Foliar produced no effect on yield. In the increased fertility block, the Trichoderma granule treatment was the only treatment that produced a significant yield increase over control. In this small trial, there are some interesting increases in yield related to the presence of microbials.

3.6 Warwick University Reports and Summary of Key Findings

The Initial Report, which focused on the fungal identifications, was received in March 2024, and the key points are presented below. The full report is enclosed as an addendum to this report (Addendum 1).

DNA was extracted from the 110 root samples It produced 3866 amplicon sequence variants (ASV) and 9914 ASV for bacteria, these sequences being analogous to individual taxa. Initial analyses were extremely interesting. Another term used below is Rank Abundance (RA). This is one of the most commonly used mathematical measures in microbiome studies and other ecological analyses and is the rank abundance curve. The rank abundance curve or the Whittaker plot is a computational measure of relative species abundance, species richness, and species evenness.

Fungal Summary

- There were significant differences in fungal diversity between locations. Across sites and age categories arbuscular mycorrhizal fungi and pathogens were both present in very low abundance within the roots. Similarly, Trichoderma spp., which are often used as plant growth-promoting inoculants, were also not detected across the samples.
- The dominant fungi detected had very low homology to characterised strains and were assigned to the Heliotales family.
- The initial analysis of the bacterial community indicated that the signatures of the types of bacteria in each of the 22 communities across sites and *Miscanthus* age classes were actually very similar.

In summary, the 22 *Miscanthus* sites had very different fungal populations, but the bacterial populations were very similar, a result that was not expected.

The categories of fungi that could be expected to be found in *Miscanthus* populations, and the actual detection levels in this survey are presented below.

Miscanthus leaf blight: *Leptosphaerulina* not detected

Wild *Miscanthus* populations *Sporisorium kusanoi, Naemacyclus culmigenus:* not detected.

Miscanthus pathogens in Mississippi *Asochyta, Alternaria, Bipolaris, Colletotrichum, Curvularia, Phoma, Pithomyces, and Septoria*: not detected.

Cereal pathogens *Fusarium sporotrichiella, Rhizoctonia solani, Fusarium poae, Sordaria fumicola*: *S. fumicola* detected in very low abundance at one sub-location.

Miscanthus root rot: *Fusarium oxysporium, F. avenaceum, Mucor hiemalis*, and *Fusarium oxysporium* detected sporadically in low abundance.

Mutualistic fungi

- *Tetracladium spp*. Endophytes: 18 ASV, 0.79 % of RA
- *Serendipita spp*. >60 ASV including *S. indica*: 0.55 % RA
- Glomeromycotan arbuscular mycorrhizal fungi: 85 ASV:0.16 % RA
- Mucoromycotinan arbuscular mycorrhizal fungi: absent

Generalist pathogens

- *Gibellulopsis nigrescens*: 0.49 % RA
- *Dactylonectria* spp: 0.36 % RA
- *Fusarium spp.*: 0.28 % RA

Dominant fungi in the *Miscanthus* microbiome

- Heliotales fungus: 2 ASV;18.2 % RA: present at all sites93 % similarity to *Psilachnum* spp.; found as plant endophytes elsewhere; potentially related to dark septate endophytes.
- *Exophiala, Trematosphaeria, Pyrenochaetopsis, Farlowiella, and Asterostroma are* all within the top 10% RA.
- [redacted].

Bacterial Summary

The analysis of the bacterial activity at the 22 locations highlighted the high relative abundance of pathways involved in the degradation of plant biomass linked to saprotrophic functions of the bacterial root microbiome. The top 20 most abundant bacterial ASV across sites used compounds such as methane as substrates. This high abundance of anaerobic fermentation functions points to an oxygen-depleted environment in the root zone of *Miscanthus*. Analysis of nitrogen cycle functions showed that aerobic and anaerobic denitrification functions were present, suggesting the presence of NH_4^+ denitrification pathways. However, no nitrogenase pathways associated with fixation of N_2 to NH_3 were detected.

- The root fungal communities showed high variability between the different plantation ages and soil types within sampling locations and were unique to each sampled location.
- The bacterial microbiome of *Miscanthus* was similar across the different plantation ages and soil types.
- Functional analysis indicated that N2 fixation was not operating in the roots of *Miscanthus* at any of the sites
- The functional analysis also indicated that the *Miscanthus* root microbiome could operate in an anaerobic environment, with evidence for methanogenesis pathways in which plant material is broken down.
- The presence of denitrifying pathways in the *Miscanthus* bacterial microbiome also pointed to the *Miscanthus* root zone being a location where denitrification of NO_3 and NH_4 to nitrous oxides could be operating
- A wide variety of pathogens were present in roots across all locations.

    - [redacted].
    - *Pyrenochaetopsis leptospora,* a fungus linked to root rots, was widely distributed across sites. Most of the pathogens were encountered sporadically and at low abundance, but they could have high relative abundance within specific fields or sub-locations within fields. Historically, *Miscanthus* has been considered a plant which has not exhibited examples of being compromised by fungal or bacterial infections. At most sample sites, the presence of root infecting fungi known to damage some grasses is interesting and should be further explored.
    - *Miscanthus* was found, in this survey, to support only a low abundance of arbuscular fungi, which have been linked to *Miscanthus* in previous studies as beneficial associations. However, these results indicated that *Periconia macrospinosa,* a dark septate endophyte, was abundant at all sampling locations, and this fungus is known to form mutualistic associations.

Addendum 1

Miscanthus **Microbial Sampling Report for Dominant Species of Fungal and Bacterial Associations, Subcontractor Warwick University**

Introduction

The rhizosphere represents the area of soil immediately adjacent to plant roots, which has a distinct chemical, physical and microbial environment to the bulk soil. There is growing recognition that the rhizosphere microbiome plays a crucial role in regulating plant health and nutrition, and unravelling the cross talk and interactions between the plant and its rhizosphere microbiome holds considerable promise to devise resilient and sustainable low input approaches to sustain crop yields (Hunter et al., 2014).

Research on the *Miscanthus* microbiome has mostly been conducted in Asia and we have limited understanding about the factors which shape the *Miscanthus* microbiome under UK and European climates and soils. Furthermore, much research on the *Miscanthus* rhizosphere has been focussed on single study sites (Chen et al., 2020, Ma et al., 2021) and glasshouse grown plants (Kane et al., 2024). Since microbiome composition is the result of complex interactions between the host, climate and soil, the relevance of these studies for the composition of the *Miscanthus* microbiome at the landscape scale is unclear.

The way in which the *Miscanthus* microbiome interacts with the host to affect its health and productivity is also largely unknown. Perennial C4 grasses may harbour endophytic nitrogen fixing bacteria in their roots, and evidence from the US suggests these could contribute 16 % of plant nitrogen (Keymer et al., 2013), however the relevance of this for UK soils is unclear. Importantly most work on the *Miscanthus* microbiome has studied only the bacterial community, and while studies indicate that *Miscanthus* roots host endophytic fungal communities including arbuscular mycorrhizal fungi (Barnes et al., 2016), there is limited understanding of the composition of these communities. Importantly, little is known of the root diseases associated with *Miscanthus*. Fusarium has been associated with negative establishment of *Miscanthus* (Thinggard, 1997), while in Italy *Miscanthus* has been infected by a root rot caused by a disease complex of *Fusarium avenaceum, Fusarium oxysporum* and *Mucor hiemalis* (Covarelli et al., 2012).

The aim of this work was to:

1. Determine the composition of the *Miscanthus* bacterial and fungal microbiome at the landscape scale
2. Investigate relationships between *Miscanthus* microbiome composition, age of *Miscanthus* plantation and soil characteristics
3. Investigate the potential for the *Miscanthus* microbiome to affect crop health including:
   a. Nitrogen fixation
   b Mutualistic symbionts including arbuscular fungi
   c. Plant pathogens

Methods

Sampling approaches

Root samples were collected from 22 *Miscanthus* fields across eight locations in the southwest of England in October 2022. The fields had been established for between 3-24 years prior to sample collection (Table 1). At each field five samples were collected following the approach described in Barnes et al. (2016). Sampling was conducted 25 m into the field to avoid edge effects. Five sampling sites were located at 20 m intervals along a transect towards the centre of the field. At each sampling site, four 150 g soil subsamples from 0-20 cm depth were taken 1m from the central position in north, south, east, and west directions and pooled together. Samples were kept cold and processed within four days of collection. Roots under approximately 2mm diameter were collected using tweezers. Healthy white roots were separated from darker senescent roots. Adhering soil was removed using three sequential washes in sterile deionised water (Hilton et al., 2021). Soil samples were sent for analysis of pH and available P, K, and Mg.

DNA extraction, sequencing and bioinformatic analysis
DNA was extracted from roots with the DNeasy PowerSoil pro Kit (Qiagen, UK). Prior to extraction samples were homogenised using a FastPrep-24 (MP Biomedicals, USA). DNA concentrations were measured by fluorometric quantification using the Qubit™ Fluorometer 3.0 dsDNA high sensitivity assay kit (Invitrogen, USA). Fungal and bacterial community sequencing and bioinformatic analysis were performed using the methods outlined in Hilton et al (2021). Fungal sequencing was conducted by amplifying the fungal ITS2 region (fITS7-ITS4), while bacterial sequencing used the V3-V4 region of the bacterial 16S rRNA gene (515F and 806R).

Demultiplexed sequences with primer sequences removed were processed using the DADA2 pipeline in Quantitative Insights into Microbial Ecology (QIIME 2.0). This removed low quality reads, chimeras and singletons. *Scikit-learn* naïve Bayes machine learning algorithm was trained on 99%-clustered Silva SSU 138 database for 16S rRNA and UNITE 2020 for ITS, and then used to assign taxonomy. Taxonomy was assigned to amplicon sequence variants. A total of 7532724 bacterial reads were obtained, and after removing mitochondrial and chloroplast sequences there were 13679 unique bacterial ASV. There were 7421780 fungal reads, and 3485 unique fungal sequences were obtained. Samples were rarefied to 3900 reads for both fungal and bacterial analyses. This depth captured the bulk of the diversity. A total of 22 samples with sequencing depth below this threshold were removed for fungi, and four for bacteria.

Data analysis
The RStudio package was used to perform analysis of similarity (ANOSIM), similarity percentage (SIMPER) and Fisher's alpha diversity analysis using the *phyloseq* and *vegan* packages. For data visualisation *ggplot2*, *ggtern*, *dplyr* and *ggpubr* packages were used. The *Rstatix* package was used for analysis of variance (ANOVA). The methods used for community assembly analyses were based on work of Stegen *et al.* (2012) and the *ape* and *picante* R packages were used. PICRUSt was used to predict the metagenome from bacterial 16SrRNA reads, while FUNGuild was used to predict fungal guilds including saprotrophs, pathogens and endophytes from fungal ITS reads.

Results
At the phylum level the *Miscanthus* root microbiome was similar across locations, comprising largely of Actinobacteriota Proteobacteria, Firmicutes, Verrucomicrobota, Chloroflexi and Acidobacteria, while the fungal microbiome consisted largely of Actinomycetes with 2-20 % Basidiomycetes (data not shown). At the class level (Figure 74a) the bacterial microbiome was dominated by alpha-Proteobacteria, Actinobacteria and Bacilli, while fungal communities were comprised of Leotiomycetes, Dothideomycetes, Leotiomycetes and Sordariomycetes, with Basidiomycota from the Agaricomycetes also represented (Figure 74b). There was no significant difference in Fisher's alpha diversity across sites, and similarly fungal community diversity was comparable across sites (Figure 74c,d). At the ASV level, ANOSIM was used to investigate

differences in community composition across sites and Non-Metric Multidimensional Scaling (NMDS) was used to visualise these relationships. ANOSIM reports the level of dissimilarity between locations (R value) and the associated level of significance. R is scaled to be within the range +1 to −1. Positive R values indicate that samples are more dissimilar between locations than within locations and negative R values report the opposite. Bacterial community composition was similar across sites, with only Launceston C possessing a significantly dissimilar community composition to 13 of the other sites and Truro D to four sites (Figure 75a,c). In contrast almost all the sites possessed significantly dissimilar fungal communities (Figure 75b,d). The top 20 most abundant bacterial ASV across sites included a Methyloligellaceae ASV, a notable group of methylotrophs (Knief, 2015), which use C1 compounds such as methane as substrates. Udaeobacter (2 ASV in the top 20) are among the most abundant bacteria in soil (Willms et al., 2016), while Actinomycete taxa were also abundant including Actinocorallia and Streptomyces.

The predicted metagenome analysis demonstrated very similar functional profiles of the bacterial communities across locations (Figure 77). This highlighted high relative abundance of pathways involved in the degradation of plant biomass, linked to saprotrophic functions of the bacterial root microbiome. Similarly high abundance of pathways linked to degradation of volatile organic compounds (VOCs) likely reflects the importance of plant and microbe derived VOCs as substrates within the plant associated microbiota. The high abundance of anaerobic fermentation functions points to an oxygen depleted environment in the root zone of *Miscanthus*, further supported by detection of methanogenesis pathways. More detailed analysis of these pathways demonstrated presence of the three major pathways of methanogenesis (hydrogenotrophic, methyloptrophic and acetoclastic, Ferry 2011) across all sites (Figure 78). However, methanotrophic processes were also detected in the predicted metagenome indicating that methantrophic activity was also present (Figure 79). Analysis of nitrogen cycle functions showed that aerobic (dissimilatory) and anaerobic denitrification functions were present and furthermore the presence of low numbers of anammox genes indicates the presence of NH_4^+ denitrification pathways (Figure 80). No nitrogenase pathways associated with fixation of N_2 to NH_3 were detected (pathway 1). While a pathway associated with nitrogenase was detected, this involves the urease biosynthesis pathway and in the absence of pathway one is likely linked to purine degradation.

The most abundant fungal ASV showed variable relative abundance both within and between sites (Figure 81). Many of the ASV showed limited taxonomic resolution and could not be assigned to genera. Notably this included the most abundant ASV, placed into the Heliotales which comprised between 0 and 30 % of average fungal abundance across most locations. Other poorly resolved ASV with limited taxonomic resolution were assigned to Claviciptaceae, Sordariomycetes, Pleosporales, Trechisporales, and Auriculariales. The Clavicipitaceae ASV was widely distributed and comprised 1-4 % of average fungal relative abundance at most sites. The other ASV with poor taxonomic resolution were encountered sporadically across locations but were abundant when they occurred. The Sordariomycetes ASV was largely confined to the three Launceston fields, where it comprised 6% average fungal relative abundance in Launceston B. The Auriculariales ASV was found at greater than 1% average fungal relative abundance at three sites and reached over 16 % relative abundance at a location within Truro C. Similarly the Trechisporales ASV02666 was rare except at Kingston D and Kingston A where it reached 17 % of average fungal relative abundance.

FUNGuild analysis showed that saprotrophic taxa comprised 15-35 % of average fungal relative abundance across sites (Figure 82a), with notably low relative abundance at Bodmin A (18 %) and Burrington A (15 %). Several of the 20 most abundant ASV were saprotrophs, including Exophiala, Cladosporium and Clohesyomyces. Four of the 20 most abundant ASV across sites were assigned as pathogens, including ASV assigned to Plectosphaerellaceae, [redacted] *herpotricha*, *Pyrenochaetopsis leptosipra* (Figure 81). Pathogens comprised between 1-11 % of fungal relative abundance across sites (Figure 82c). [redacted] ASVs in particular was widely distributed and locally abundant, with 3 ASV detected within the top 20 most abundant pathogens (Figure 83). *O. herpotricha* ASV00801 was the most abundant and widely distributed of these, and found at all sites, reaching 11 % of fungal relative abundance at Bodmin A. *O. herpotricha* ASV 00733 was also found at most locations, but at lower abundance, reaching 5% of average fungal relative abundance at Bodmin A. [redacted] was found sporadically and at low abundance across sites, except at Launceston A where it comprised over 5% of the average fungal relative abundance. *Pyrenochaetopsis leptospora* was also detected at almost all sampling locations, comprising over 2% average fungal relative abundance at 11 of the sites.

Paraphaeosphaeria angularis, *Devriesia sp., Plectosphaeralla* and *Tetraplosphaeria* sp. were also detected across a high proportion of sites, but were detected at low relative abundance, although this exceeded 1% of pathogen at a number of sub locations (Figure 83). The remaining pathogen ASV, including*, Gaeumannomyces hyphodioides, Paraphoma* sp., *Tetracladium* sp, *Scytalidium circinatum, Hymensoscyphus peruni* and *Paraphaeosphaeria* sporulosa had localised distributions. However they could be abundant at sub locations within fields, with *Scytladium circinatum* reaching over 8% of pathogen relative abundance at a sub location at Fennington A and *Paraphoma* sp reaching over 10.5 % of pathogen relative abundance at a sub location at Kingston C.

Two of the 20 most abundant ASV were assigned to the fungal endophyte *Periconia macrospinosa* (Figure 81). Endophytes were detected at all sites, comprising over 4% of fungal relative abundance at 11 sites, and were particularly abundant at Fennington A and Salisbury A (Figure 82b). However, endophytes were only sporadically encountered at sub locations within Burrington C, Launceston A, and at low abundance. *Periconia macrospinosa* ASV02395 and 02397 were found at most sites, where they comprised up to 15 and 11 % respectively of fungal relative abundance. *Periconia* spp. is a widely distributed dark septate endophyte found in grasses and other plants (Mandyam et al., 2012), which are typically considered to be mutualistic fungi, but have been reported to be pathogens in some circumstances (Sarkar et al., 2019). *Dokmaia* was also widely distributed, detected at 20 sites, and reaching over 1% relative abundance at Bodmin B, Fennington B, Truro A and Truro B. The remaining endophytes were detected infrequently and at less than 0.5 % of fungal relative abundance except in sporadic field sub locations. Arbuscular mycorrhizal fungi were detected rarely in the microbiome.

There were no significant correlations between age of *Miscanthus* plantation and the relative abundance of saprotrophs, pathogens or endophytes. Relative abundance of the pathogen *Tetraplosphaeria* sp was significantly positively correlated with age of the plantation (r=0.30) while the reverse was true for *Dendryphion* (r=0.25). Soil pH was negatively correlated with relative abundance of [redacted] (r=0.5 and 0.3 respectively). None of the endophytes were correlated with age of the plantation. Relative abundance of *Periconia macrospinosa* 02397 was positively correlated with soil pH (P=0.25).

The assembly processes operating in the root zone were also investigated (Figure 85). This showed that the abundant members of the microbiome were subject to different assembly processes than the rare taxa. For rare bacteria communities were largely shaped by stochastic drift (random fluctuations in composition independent of environment or selective pressures), while for the common and abundant taxa dispersal limitation (ability to spread between locations) also shaped the community. For fungi common and rare taxa were largely shaped by dispersal limitation and drift, with variable selection (associated with similarity in environment, resources or traits across locations) shaping common communities. In contrast abundant communities were shaped by homogenous selection (where similar environmental conditions or selective pressures act across locations) with far lower contributions of drift or variable selection to assembly. However, there were some locations with distinct drivers of community assembly, Salisbury A and B for the abundant bacterial community and Durston A for the abundant fungal community. Assembly processes were not correlated with age of plantation or soil pH.

Conclusions

1. The bacterial microbiome of *Miscanthus* is highly conserved across the landscape even across different plantation ages and soil types.
2. Functional analysis indicated that N2 fixation was not operating in the roots of *Miscanthus* at any of the sites
3. Functional analysis indicated that the *Miscanthus* root microbiome could be operating under an anaerobic environment, with evidence for methanogenesis pathways. However methanotroph activity was also detected and net release of CH_4 would depend on the relative activity of these two pathways.
4. The presence of denitrifying pathways in the *Miscanthus* bacterial microbiome also points to the *Miscanthus* root zone being a location where denitrification of NO_3 and NH_4 to nitrous oxides could be operating
5. The root fungal microbiome was unique to each location sampled
6. Root fungal communities showed high variability between and within sampling locations
7. Many of the most abundant fungi found in the root zone showed low similarity to described species and so their functional significance is unclear
8. [redacted]
9. Most pathogens were encountered sporadically and at low abundance, but they could have high relative abundance within specific fields or sub locations within fields
10. *Miscanthus* was found to support low abundance of arbuscular fungi, which have been linked to *Miscanthus* in previous studies. Instead *Miscanthus* appears to form dark septate endophyte mutualistic associations with *Periconia macrospinosa* which was abundant at all sampling locations
11. Similar community assembly processes operated across the landscape irrespective of soil type and plantation age, so the factors which shape communities were largely the same across the sites.

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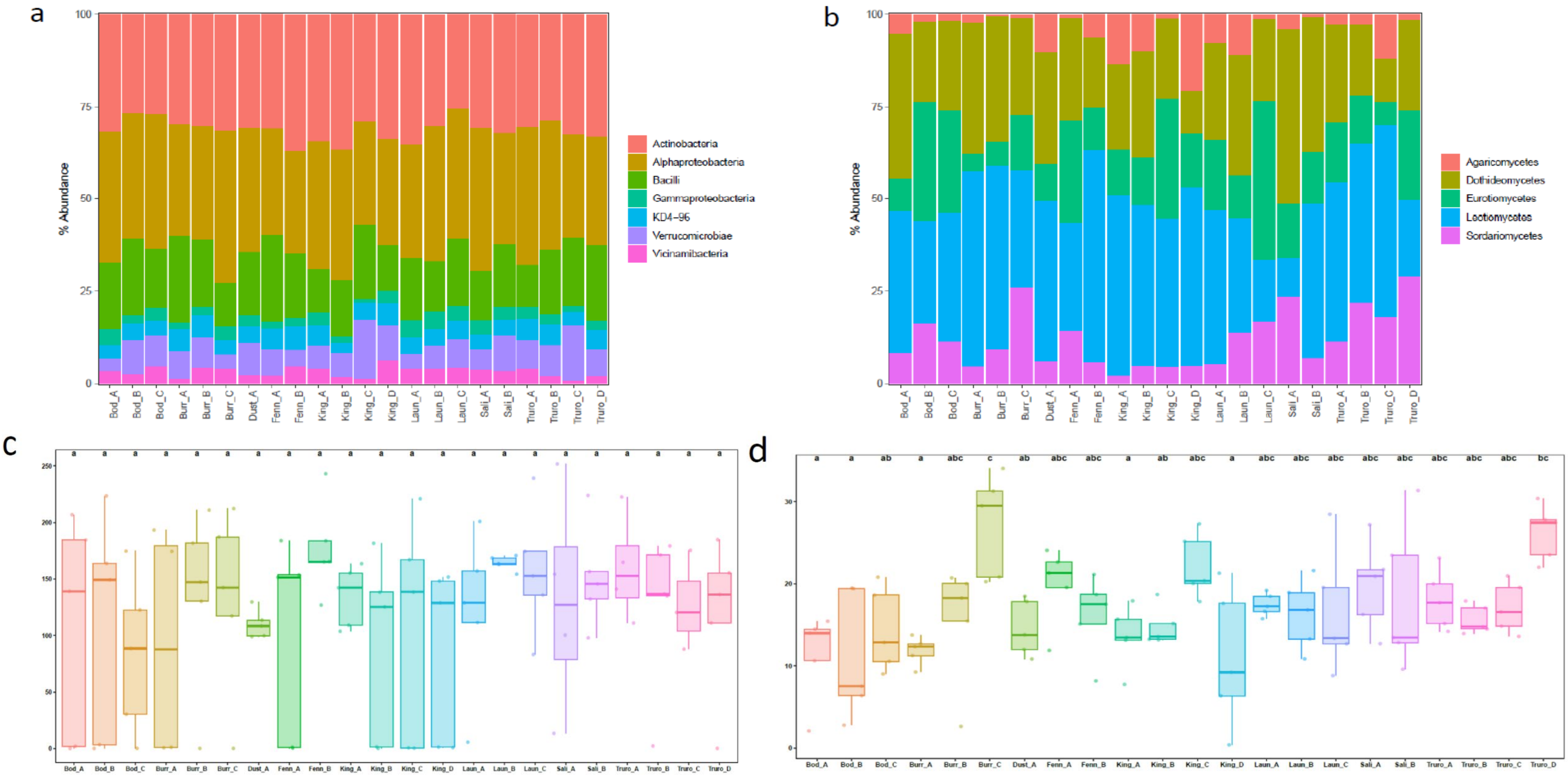


Figure 74: *Miscanthus root microbiome diversity and composition across sampling locations. (A) Relative abundance of bacterial classes; (B) Relative abundance of fungal classes; (C) Bacterial diversity; (D) Fungal diversity. Different letters indicate significant differences (P<0.05).*

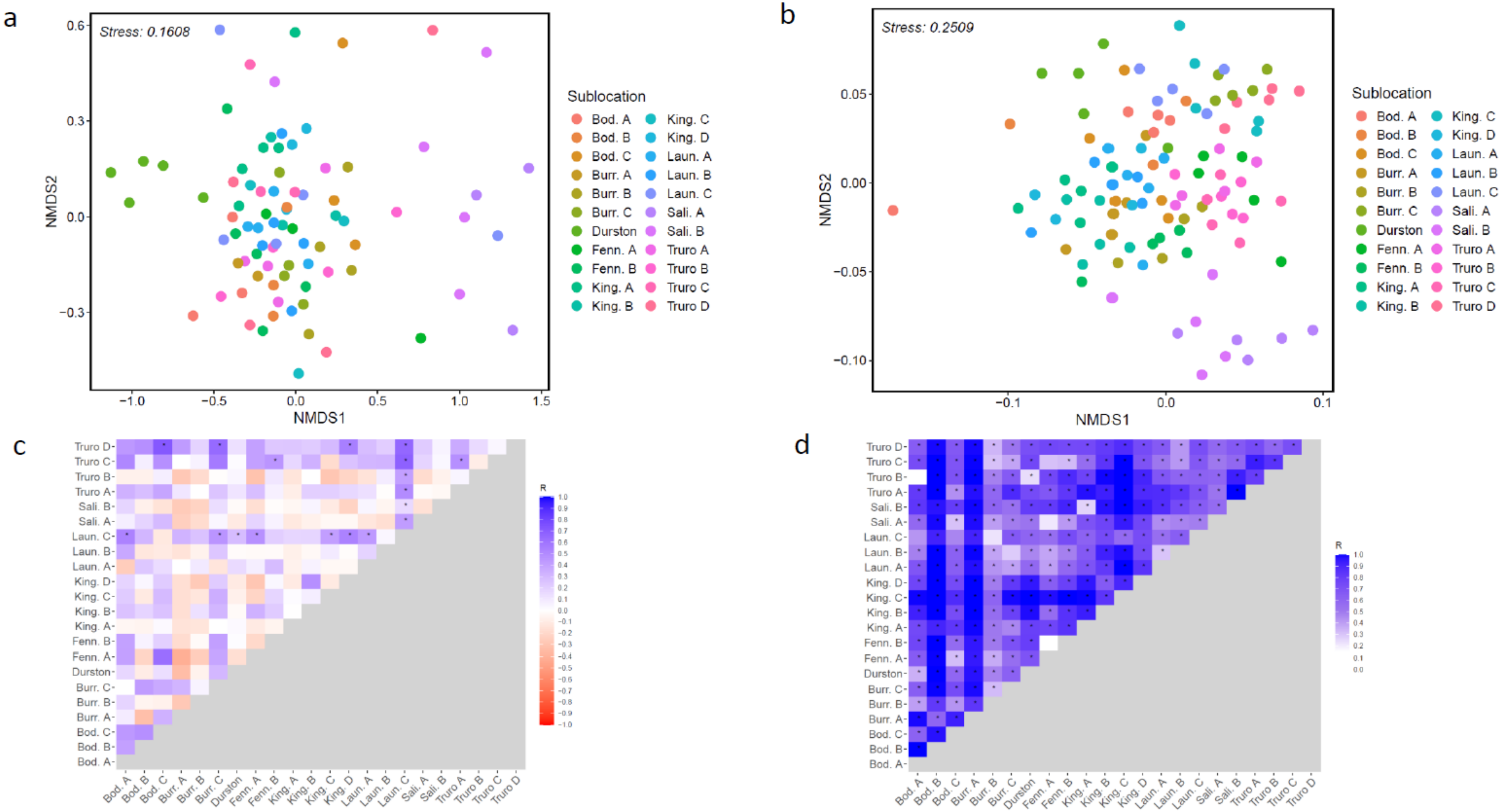


Figure 75: *Comparison of Miscanthus microbial community composition across sampling locations non-metric multidimensional scaling analysis of bacterial (A) and fungal (B) communities, Analysis of Similarity of bacterial (C) and fungal (D) communities. * Indicates significant difference P<0.05.*

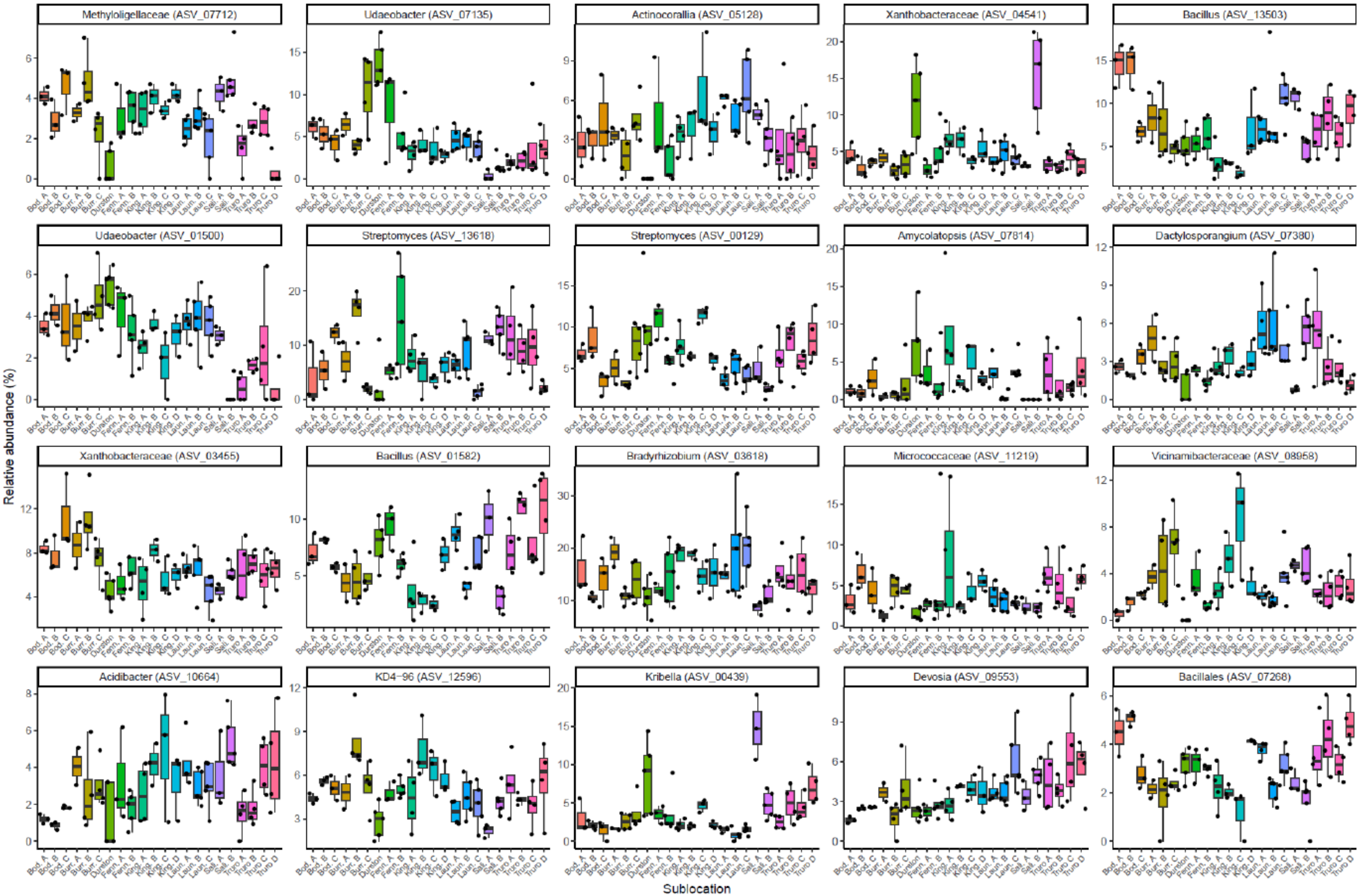


Figure 76: *Box plots showing the distribution of the 20 most abundant bacterial ASV across sampling locations.*

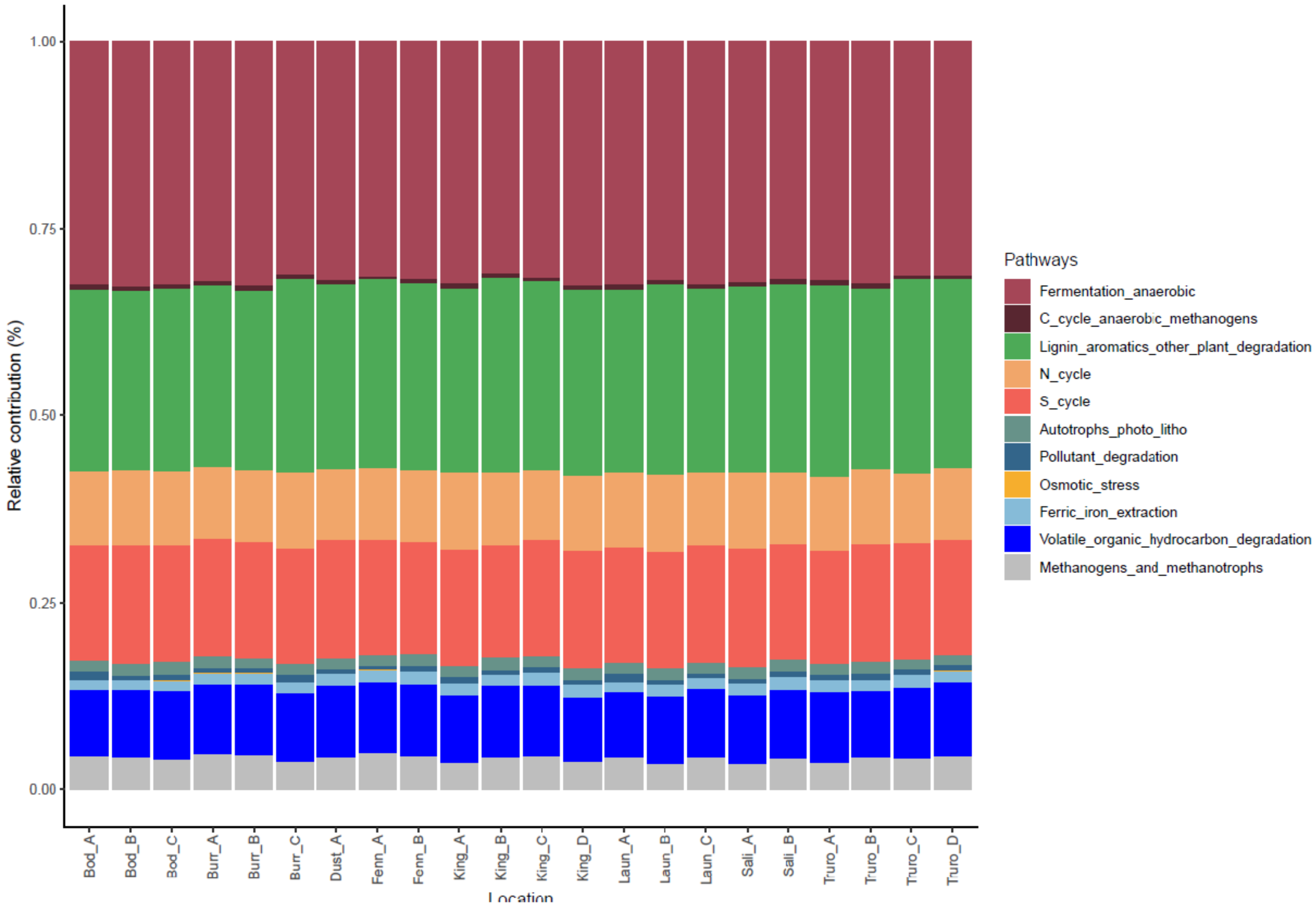


Figure 77: *PICRUSt analysis of predicted functional pathways in the Miscanthus bacterial microbiome across sampling locations.*

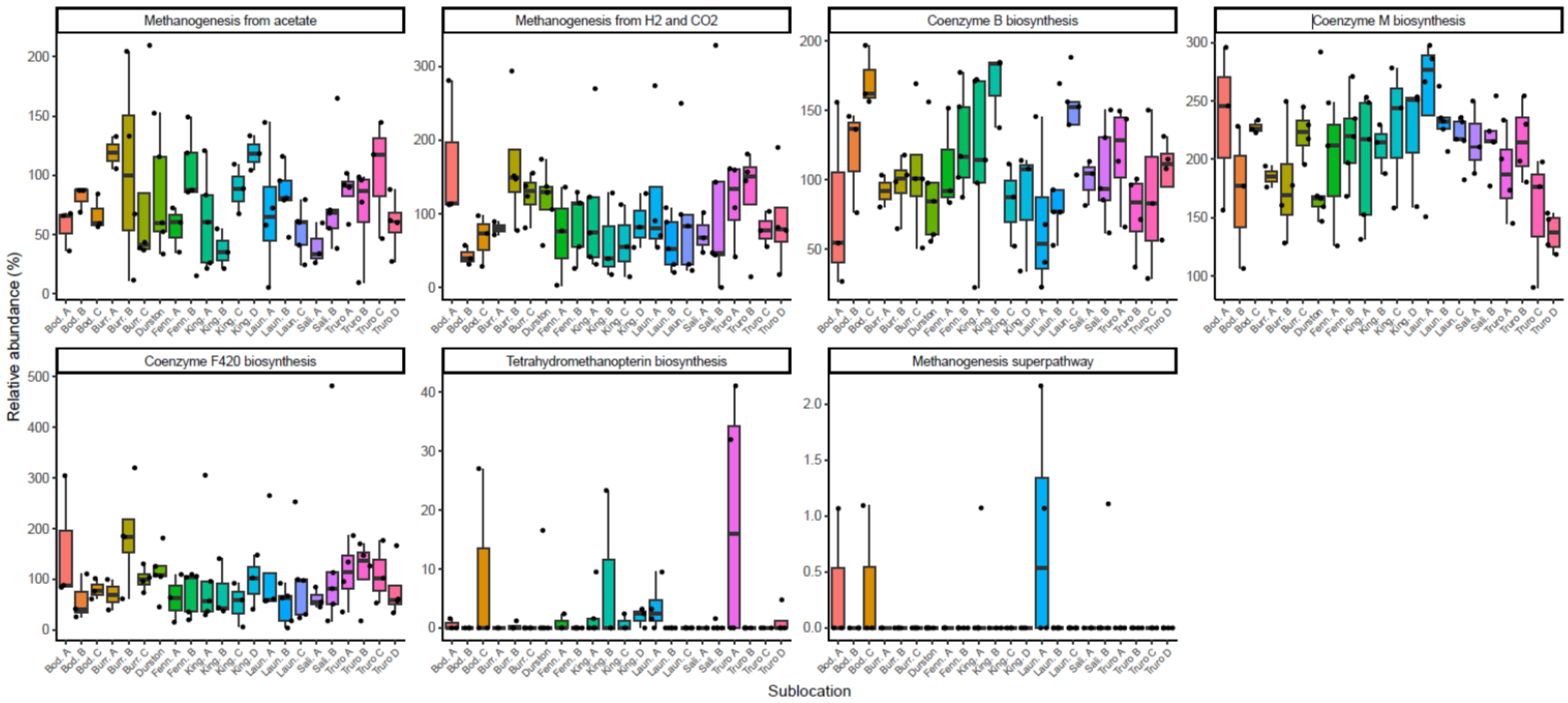


Figure 78: *PICRUSt analysis of predicted methanogenesis pathways in the Miscanthus bacterial microbiome across sampling locations.*

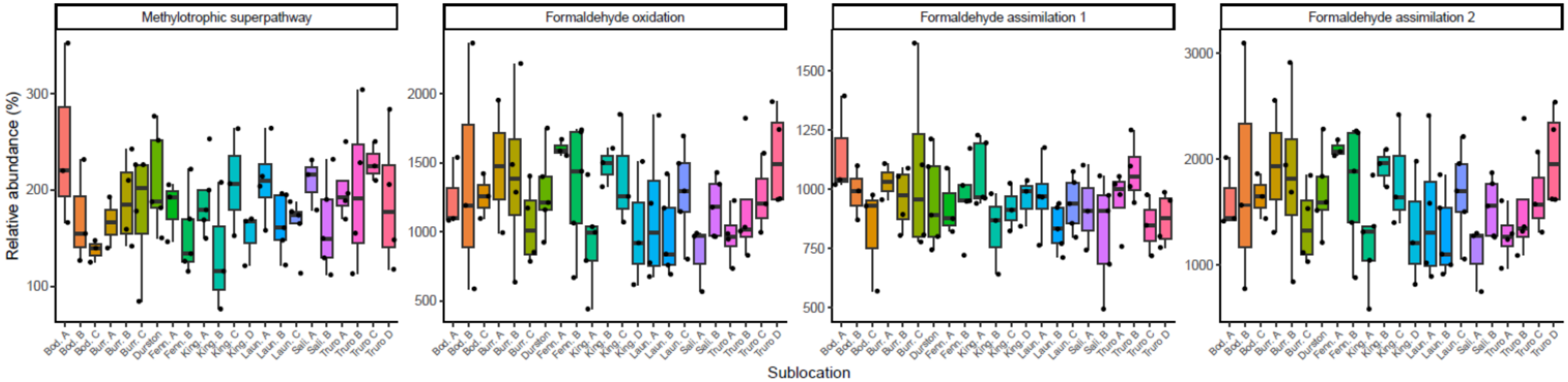


Figure 79: *PICRUSt analysis of predicted methanotrophic pathways in the Miscanthus bacterial microbiome across sampling locations.*

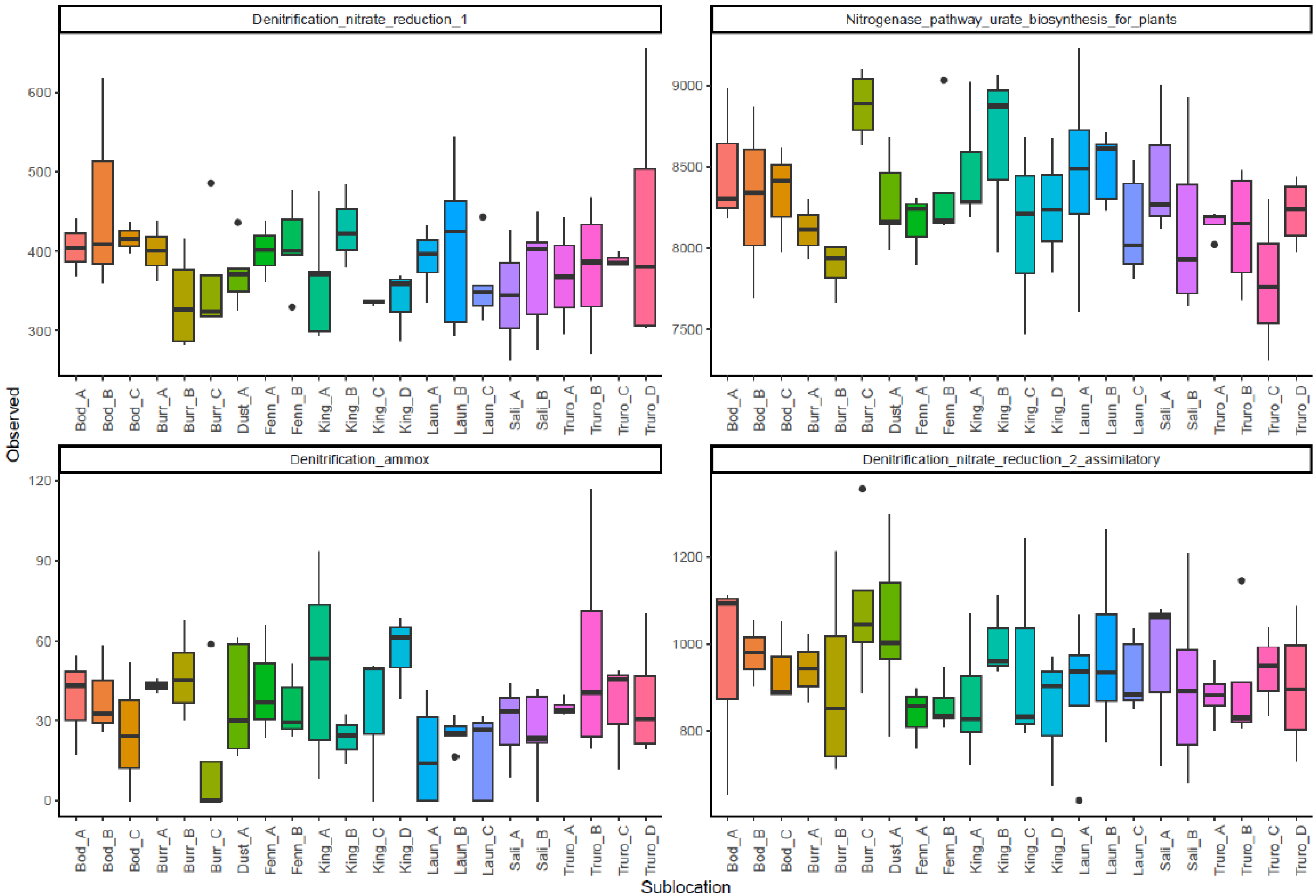


Figure 80: *PICRUSt analysis of predicted nitrogen cycling pathways in the Miscanthus bacterial microbiome across sampling locations.*

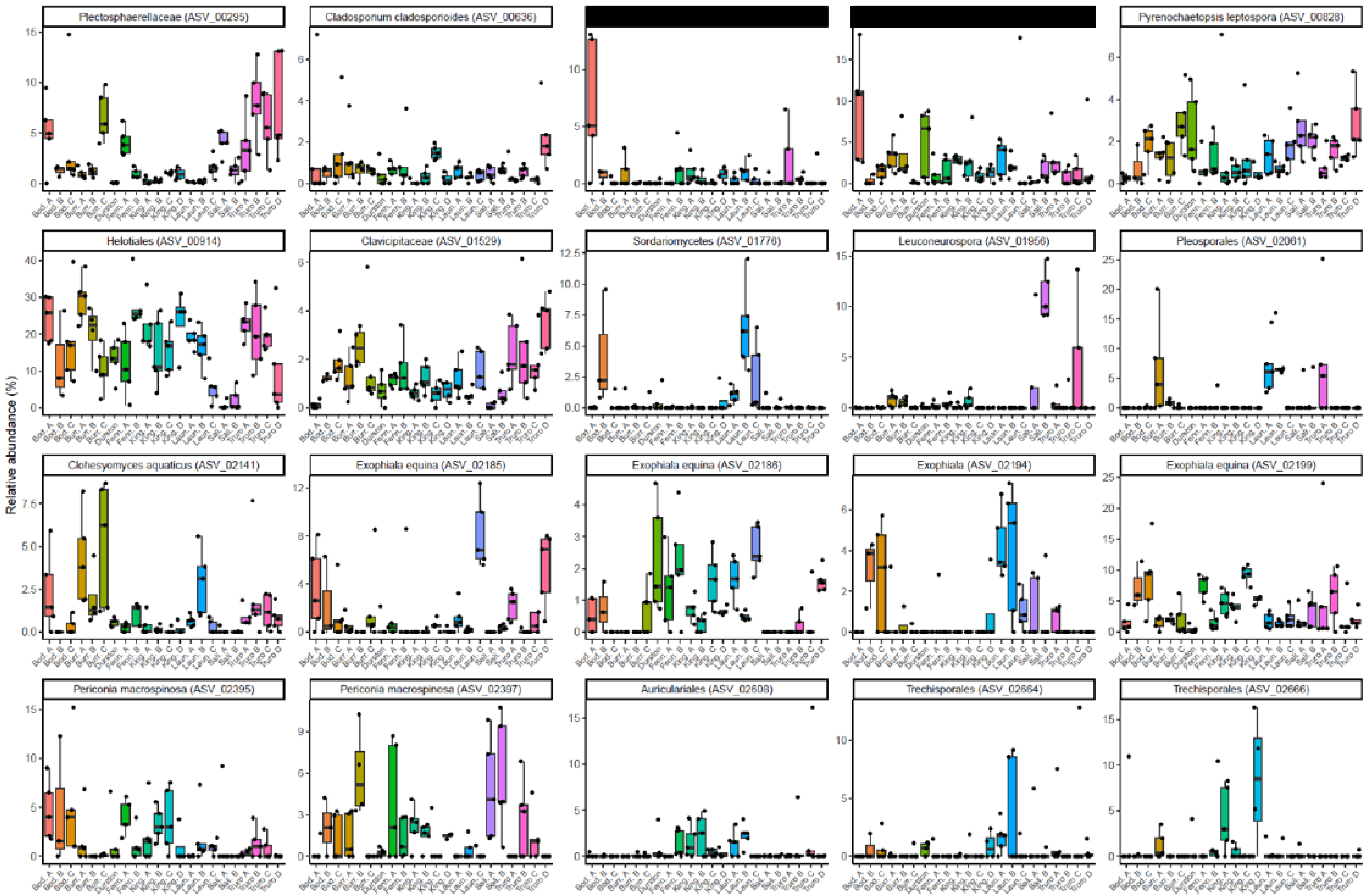


Figure 81: *Box plots showing the distribution of the 20 most abundant fungal ASV across sampling locations.*

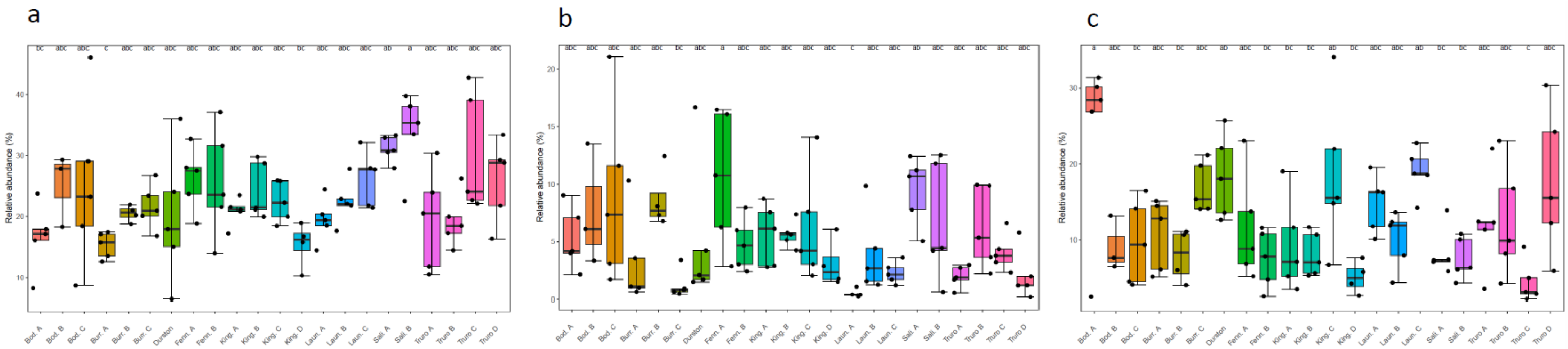


Figure 82: *Box plots showing the relative proportions of saprotroph (A), endophyte (B), and pathogen (C) fungal guilds across sampling locations. Different letters indicate significantly differences (P<0.05).*

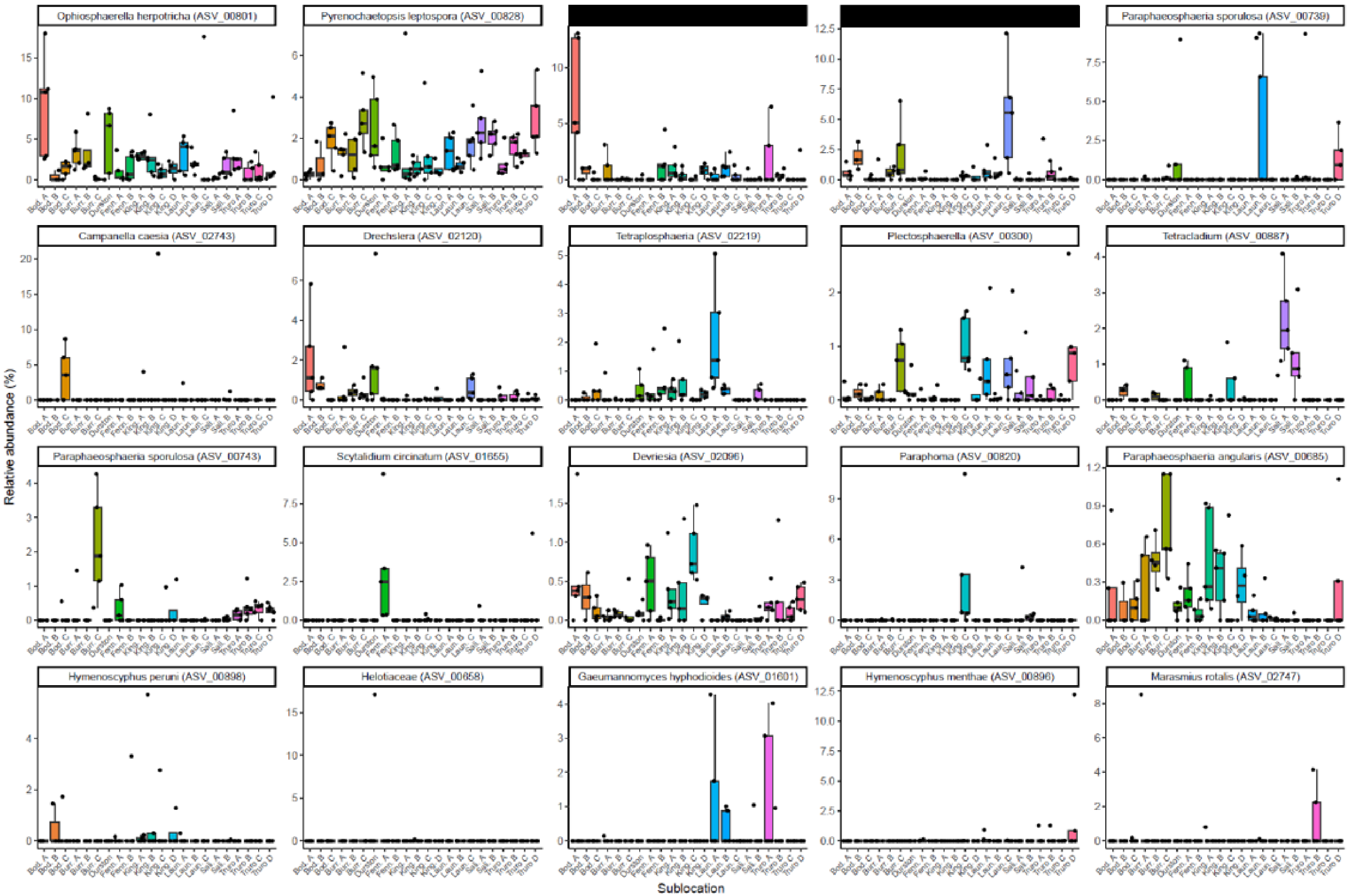


Figure 83: *Box plots showing the distribution of the 20 most abundant fungal pathogens across sampling locations.*

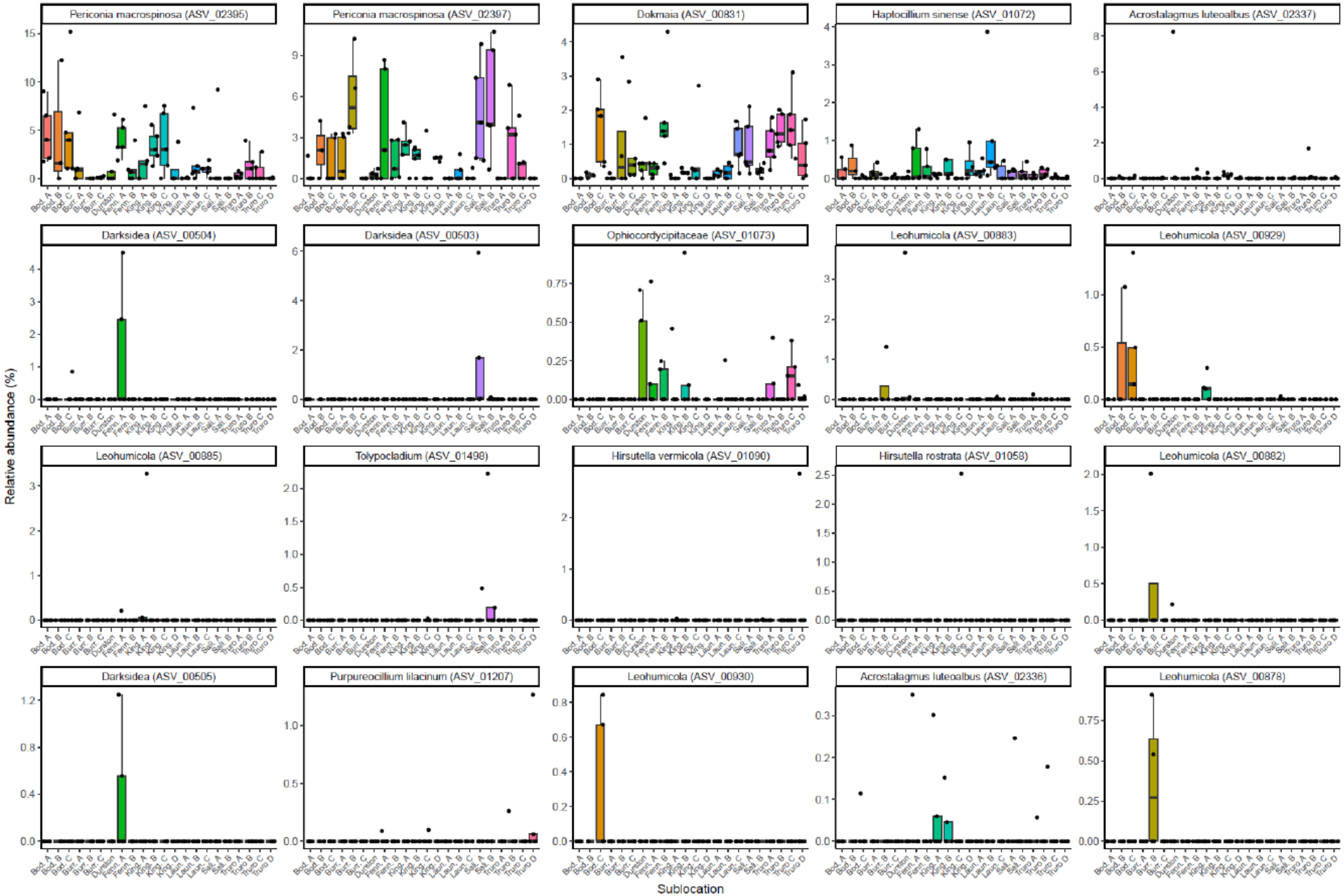


Figure 84: *Box plots showing the distribution of the 20 most abundant fungal endophytes across sampling locations.*

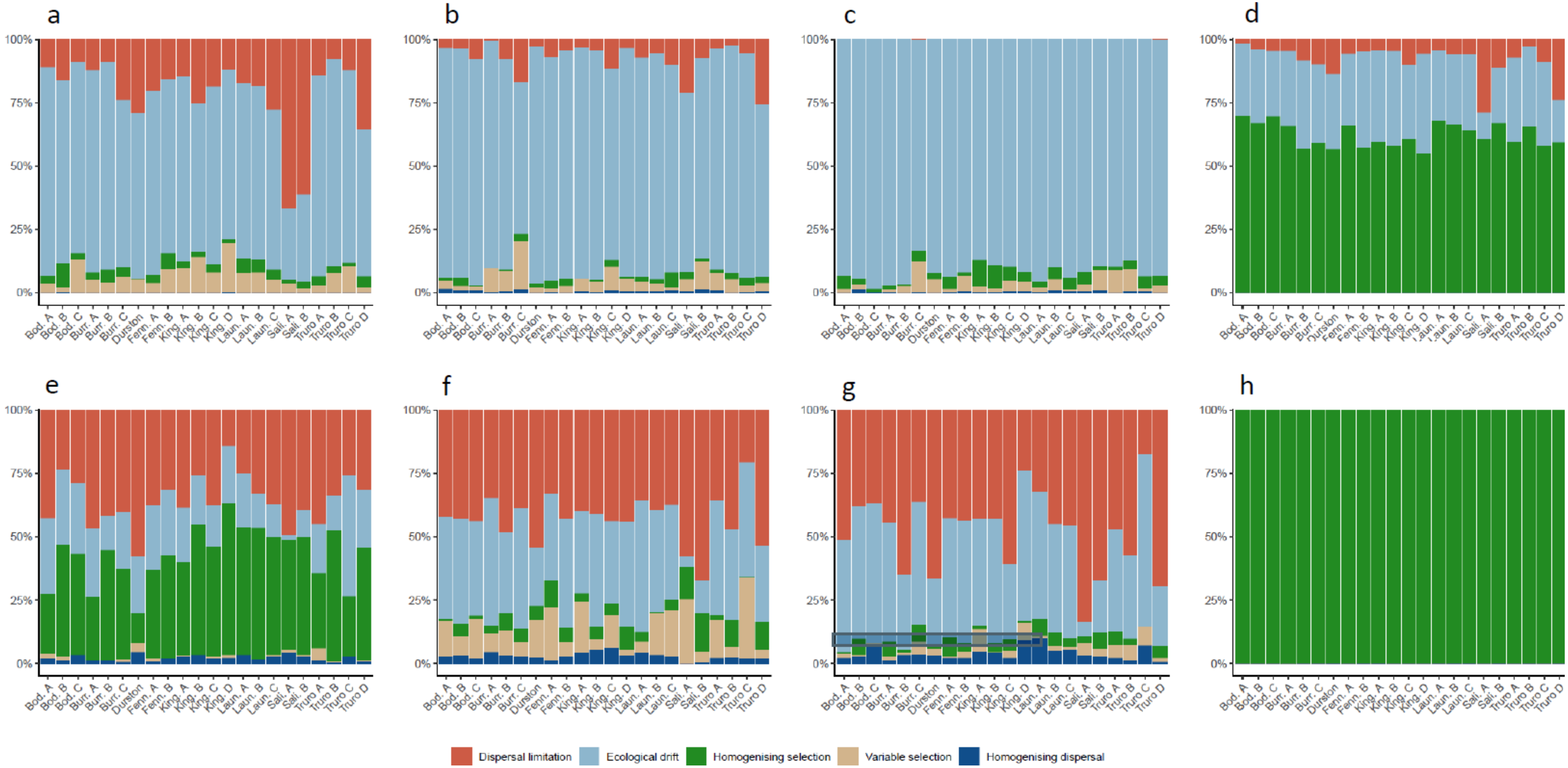


Figure 85: *Deterministic and stochastic processes associated with assembly of the Miscanthus root microbiome. (A) Abundant bacterial ASV >0.01 of community abundance; (B) Common bacterial ASV 0.01-0.001% of community composition; (C) Rare bacterial ASV < 0.001% of community composition; (D) All bacterial ASV combined; (E) Abundant fungal ASV >0.03% of community abundance; (F) Common fungal ASV 0.03-0.003% of community composition; (G) Rare fungal ASV < 0.003% of community composition; (H) All fungal ASV.*

Annex 4 – Supporting information for WP 4, Paludiculture and contaminated land.

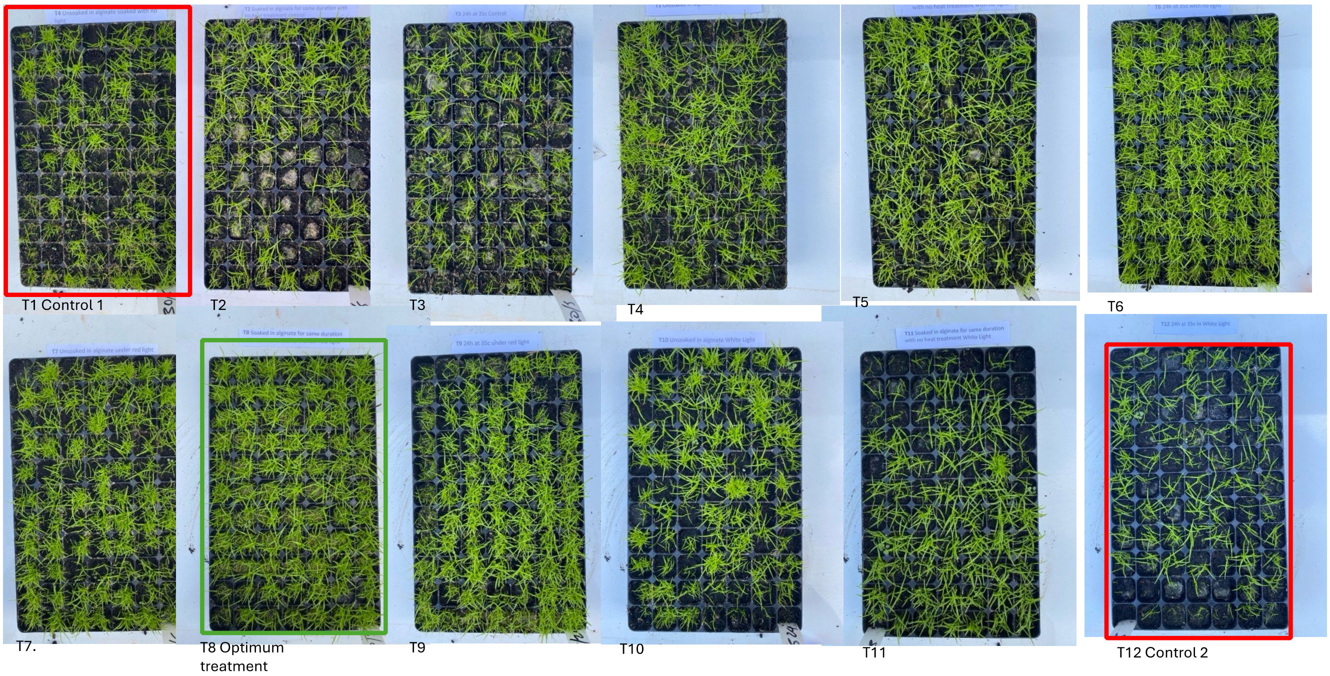


Figure 86: *Vigour of Typha germination in cell trays, the seeds having had different pre-treatments prior to sowing of hydration, thermal and light spectrums. Control treatments with red boxes, and optimum treatment (assessed as % germination x vigour) with green box.*

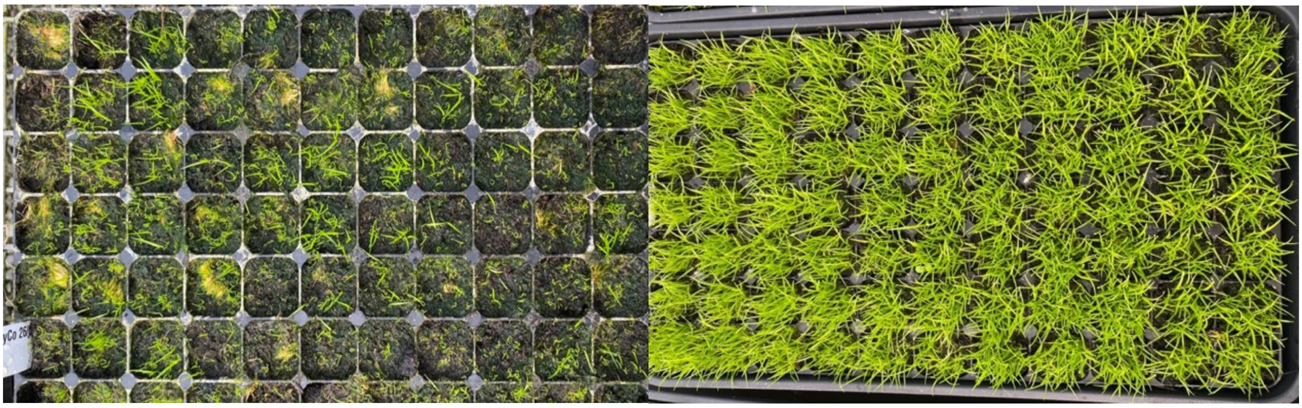


Figure 87: *Vigour of Typha germination in cell trays, the seeds having had different pre-treatments prior to sowing of hydration, thermal and light spectrums. Comparison of control treatment (left) and optimum treatment (right).*

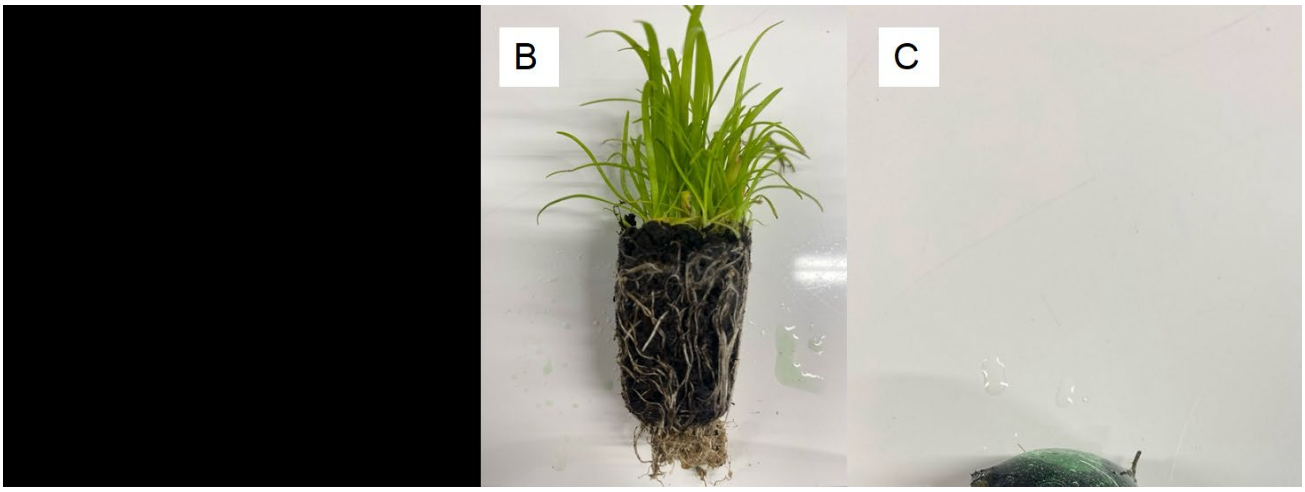


Figure 88: *Types of Typha propagules developed for commercial planting: (A) Balls; (B) Plug plants; (C) CEEDS™.*

Figure 89: *Methods of planting Typha propagules (D) Drone for balls; (E) Manual planting for plug plants; (F) Automated planter for CEEDS™.*

Table 13: *Elemental analysis of soils from different trial sites used to grow Miscanthus plants.*

Element	Symbol	mg/kg on a dry matter basis					
		Taunton (Control)	Wheal Maid 1	Wheal Maid 2	Wheal Maid 3	Wheal Maid 4	Bridford
Aluminium	Al	8,121	7,734	416	5,297	20,242	2,703
Arsenic	As	13	785	1,416	1,486	4,245	262
Iron	Fe	12,894	41,908	161,522	33,582	76,048	40,934
Potassium	K	2,490	1,129	228	1,506	3,137	1,207
Magnesium	Mg	2,059	831	0	1,065	3,131	178
Sulphur	S	234	4,239	136,488	2,951	1,621	4,844
Copper	Cu	10	52	65	129	1,588	298
Lead	Pb	23	58	506	198	979	6,285
Barium	Ba	53	73	7	50	102	1,697
Zinc	Zn	41	41	138	52	356	876

Figure 90: *Images of six soil types (left), (right) plant stress (Miscanthus) growing in Wheal Maid 2 soil.*

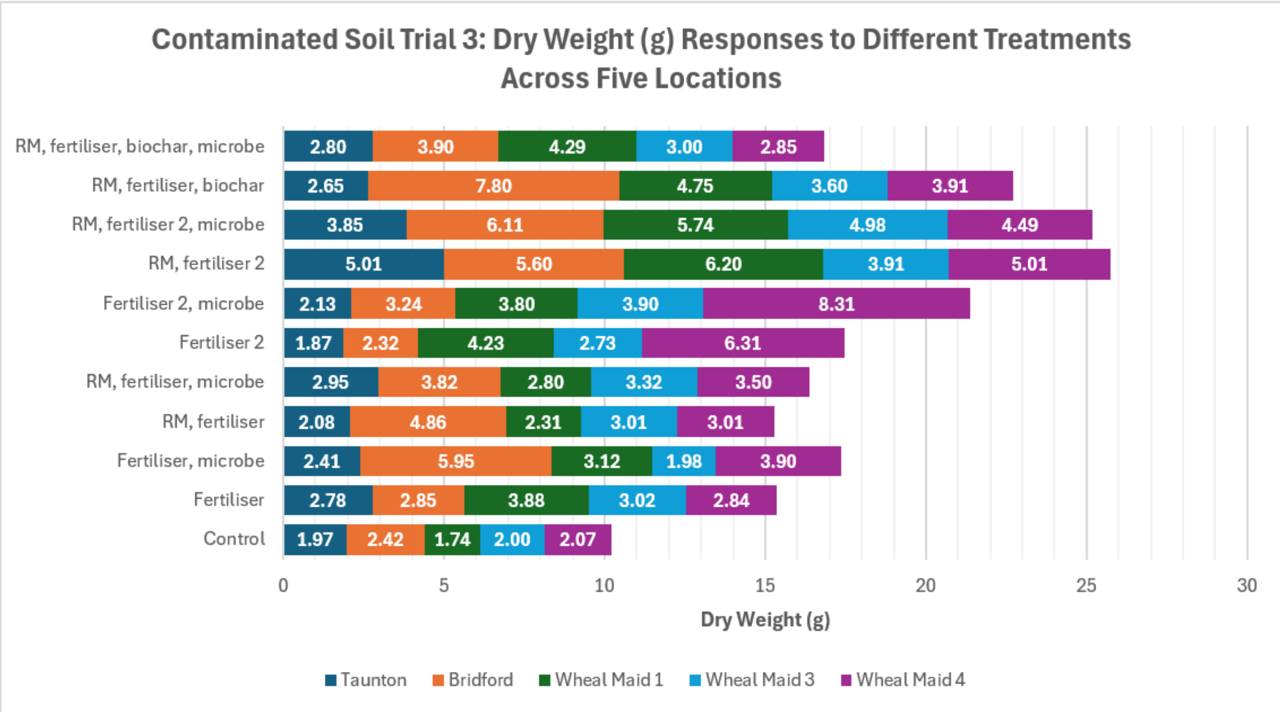


Figure 91: *Overall yield responses to the 11 treatments applied to the five soils. Results are presented with the five soil types grouped within the individual application treatments, illustrating very clearly the benefits generated from fertiliser applications (RM represents remedial product applied to mitigate the effect of the contaminated land).*

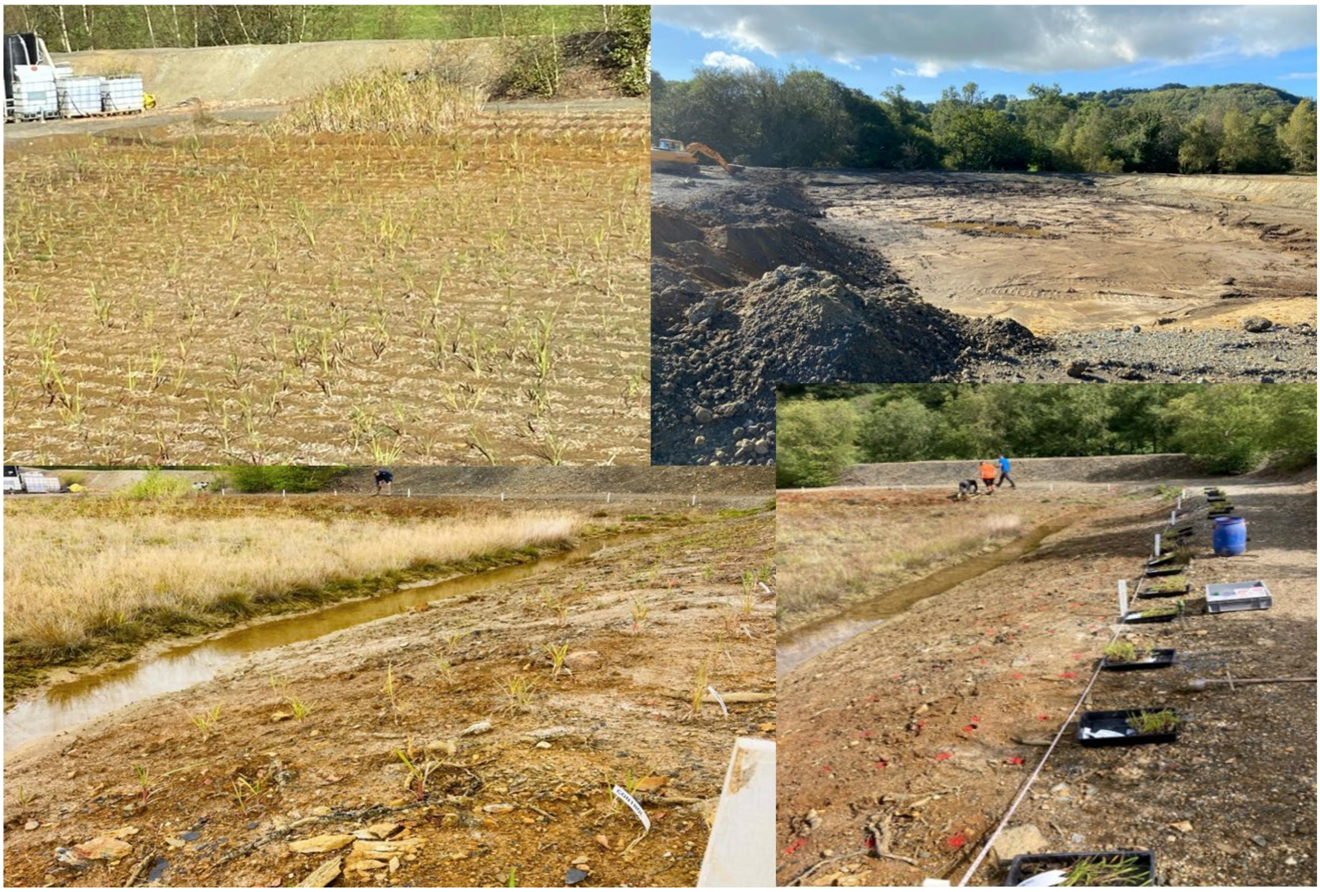


Figure 92: *Overview of the Bridford mine and the planting activities of crops on the site.*

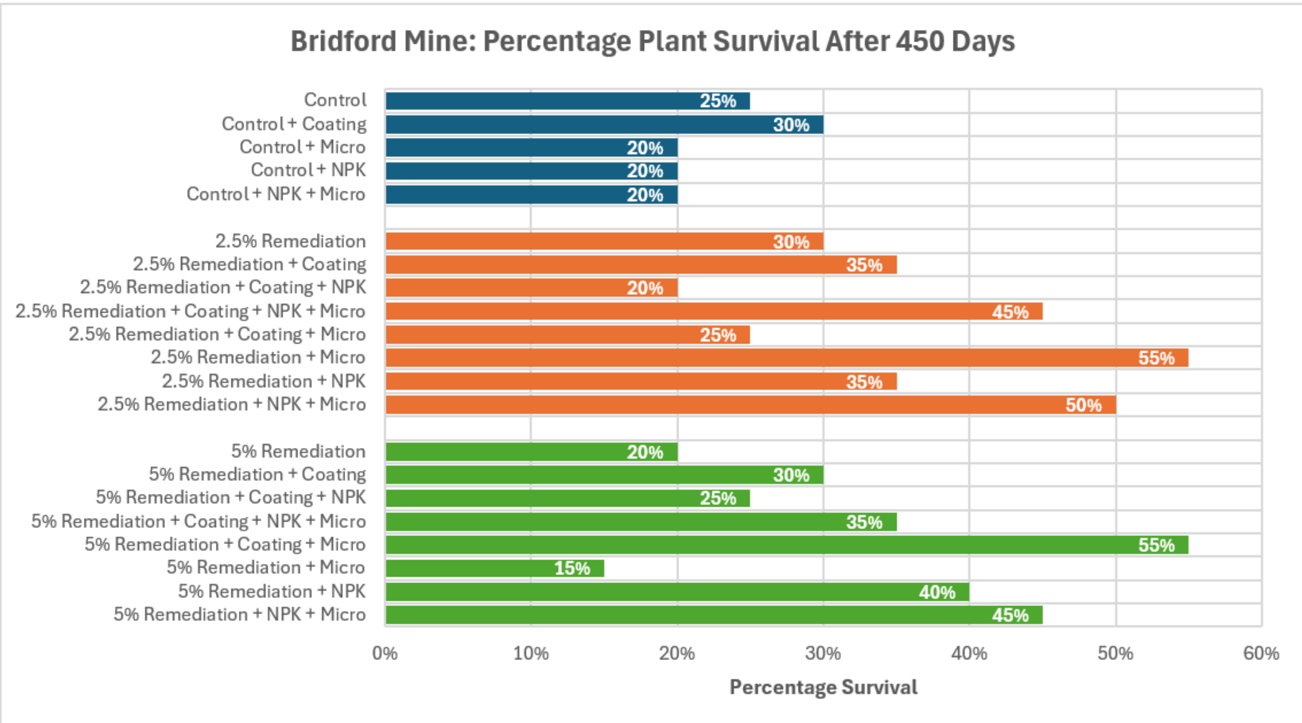
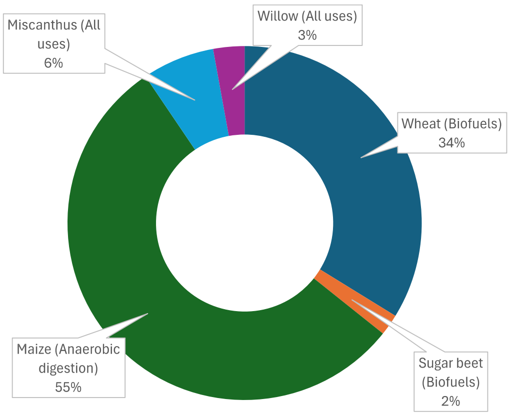


Figure 93: *Percentage establishment levels after 450 days in mine soil.*

Annex 5 – Supporting information for WP 5, Commercialisation.



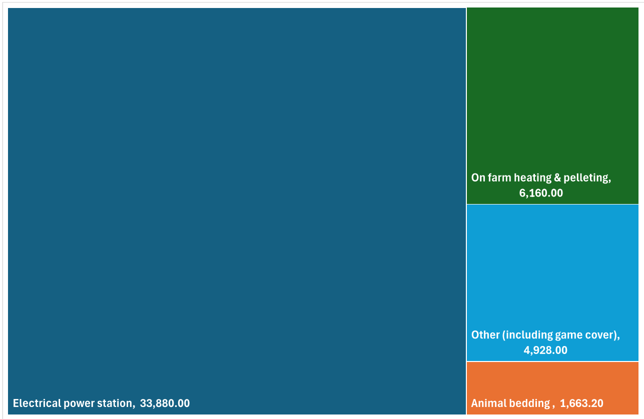


Figure 94: *Left: Breakdown of % allocation by space of the 133,000ha of energy crops grown in the UK by crop type. Right: Estimated split of Miscanthus production in the UK going into different end uses, of energy and alternatives.*

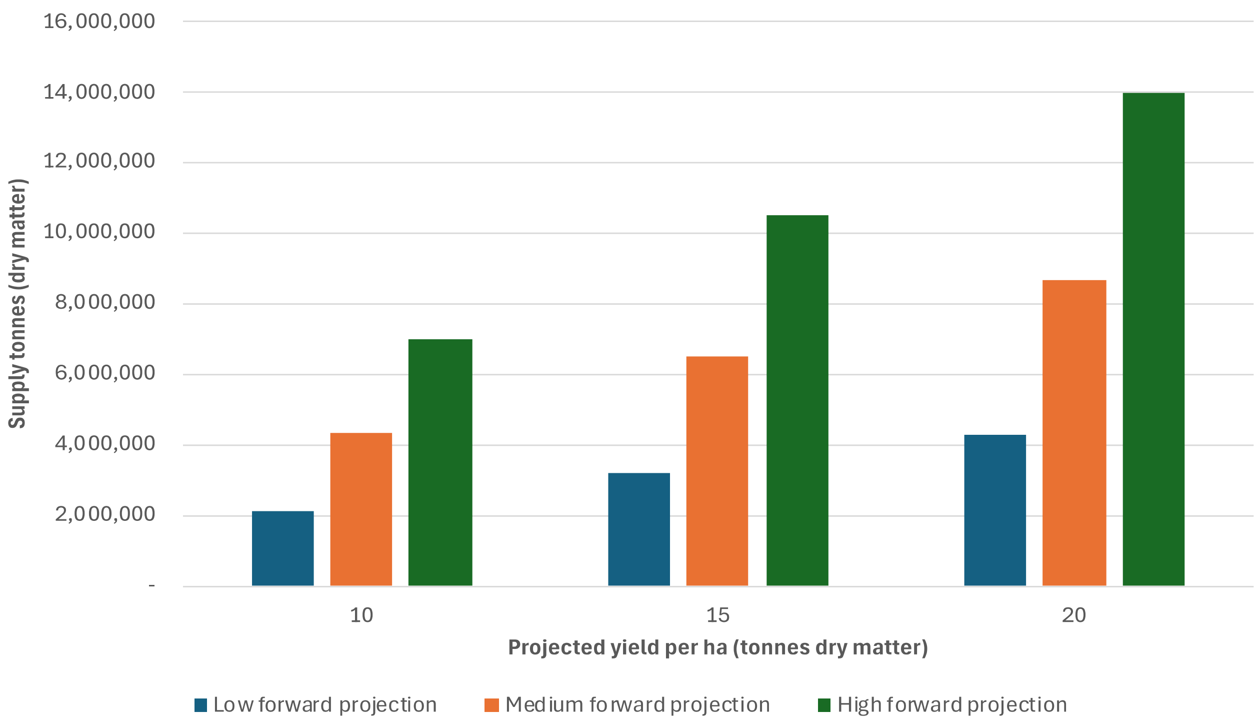


Figure 95: *Forward projections of energy crop production potential in the UK from perennial grasses using three planting area projections (214,000, 435,000, and 700,000ha total growing area), and three levels of average annual yield of 10, 15, and 20t per ha dry matter.*

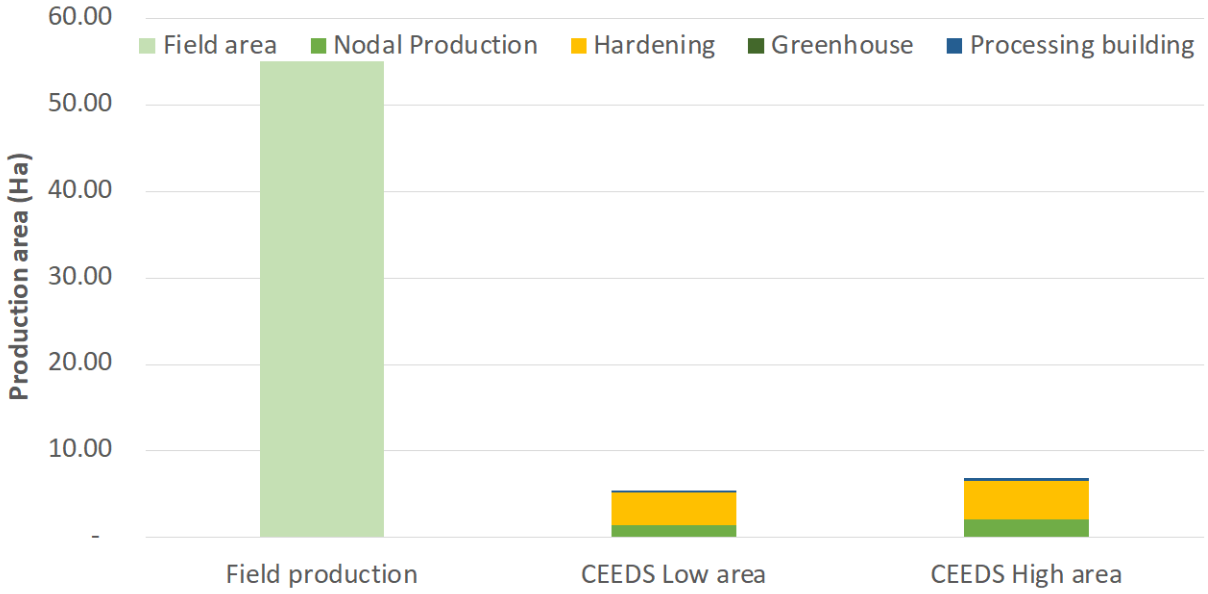


Figure 96: *Projected range of production area required for a 1,000ha output per year CEEDS™ Bio-factory for rhizome propagated crops such as Miscanthus. Production areas broken down into different components and compared to conventional field propagation of Miscanthus.*

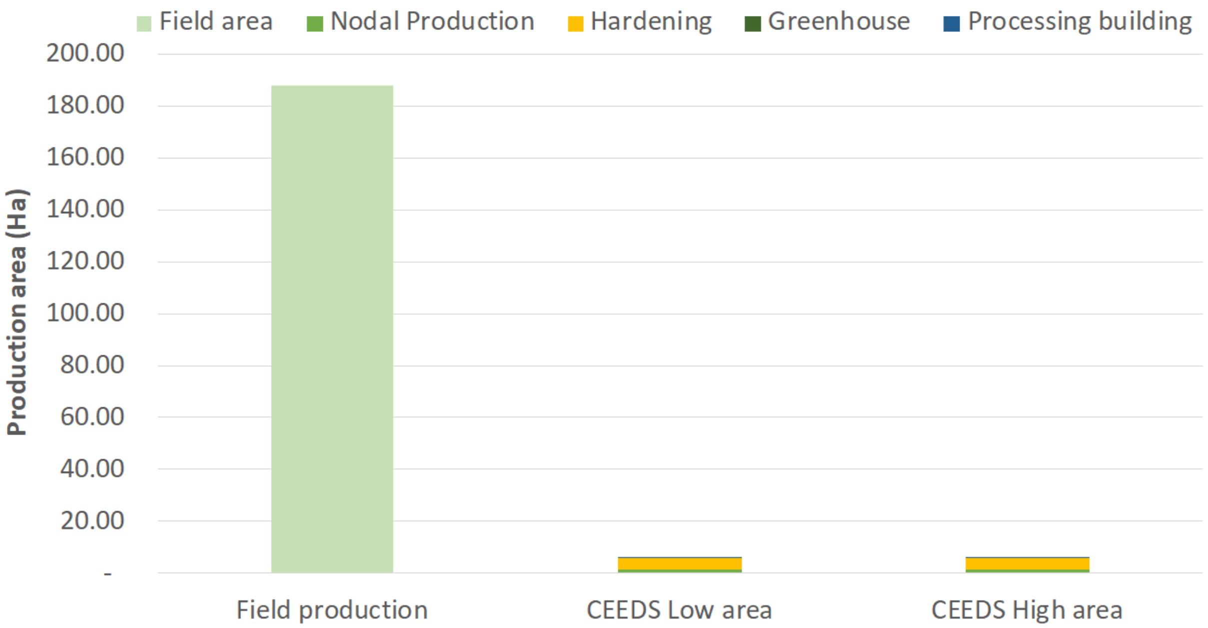


Figure 97: *Projected range of production area required for a 1,000ha output per year CEEDS™ Bio-factory for stem propagated crops such as Energy cane. Production areas broken down into different components and compared to conventional field propagation of Energy cane.*

Figure 98: *Images of initial design CAD drawings of CEEDS™ field planter (left), and final manufactured machine (right).*

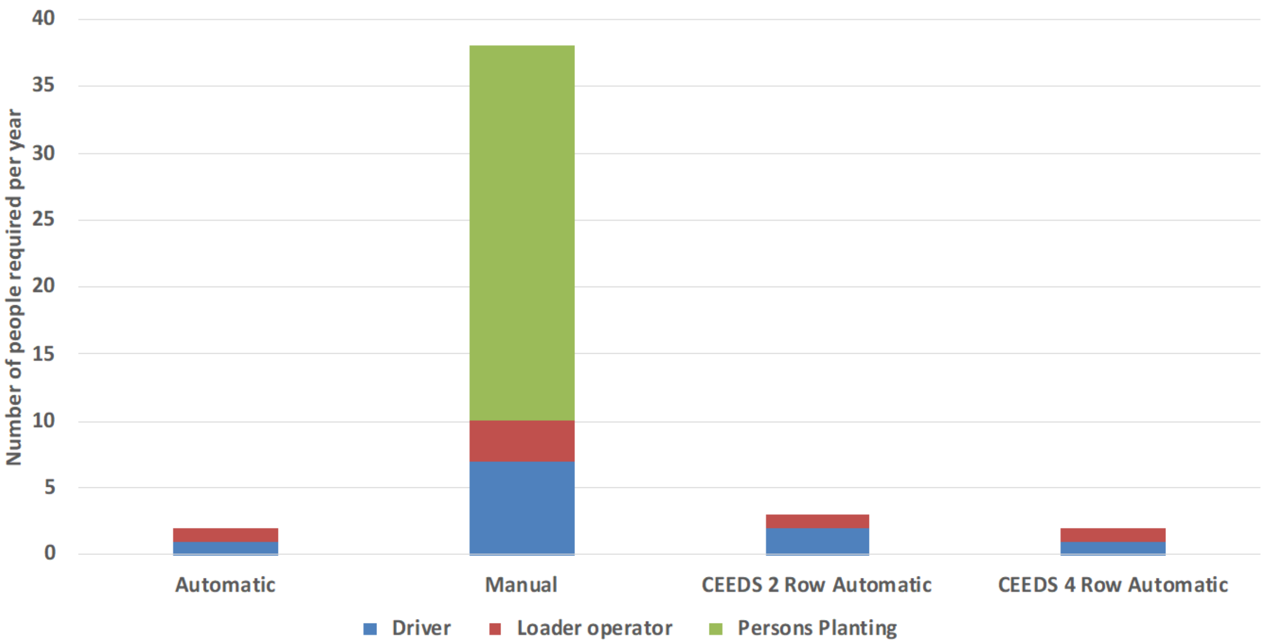


Figure 99: *Number of people required in a typical annual spring planting window to plant 1,000ha of energy crop (Miscanthus) based on four planting options of automatic rhizome planter, manual precision planter (4 row) and CEEDS™ planter (2 row and 4 row).*

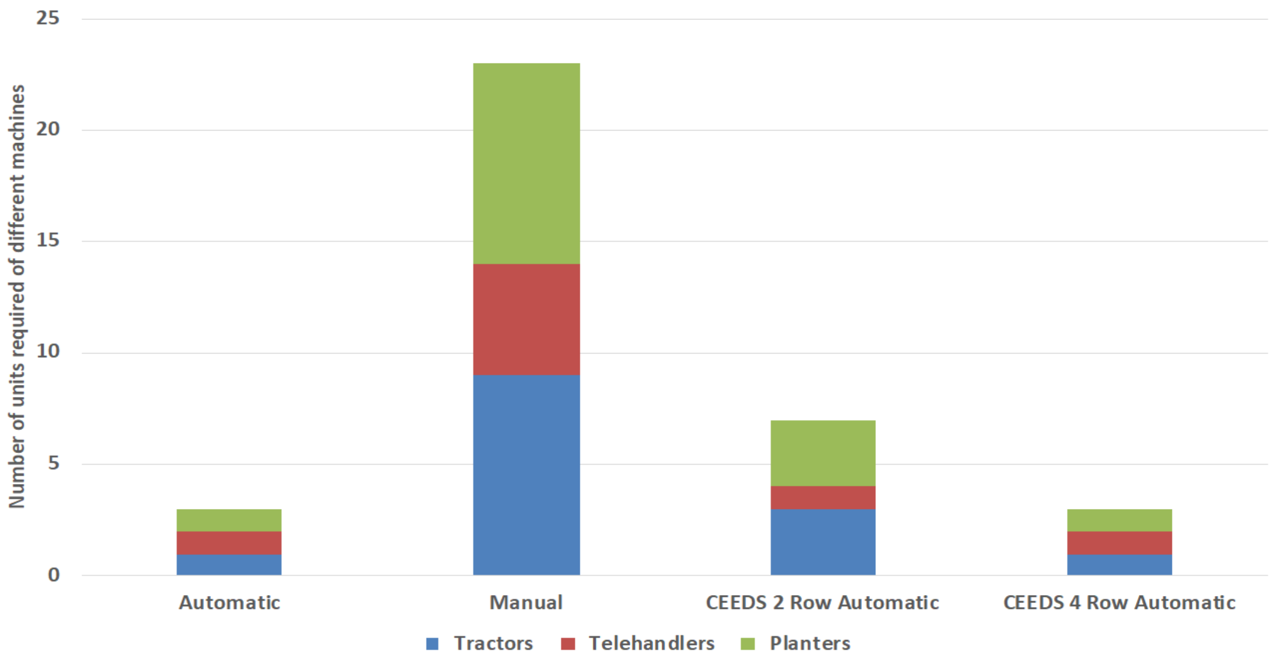


Figure 100: *Number of machinery units required (of tractors, telehandlers, and planters) in a typical annual spring planting window to plant 1,000ha of energy crop (Miscanthus) based on four planting options of automatic rhizome planter, manual precision planter (4 row) and CEEDS™ planter (2 row and 4 row).*

Annex 6 – Glossary

Glossary	
Term	**Definition**
Anaerobic Digestion	A biological process that breaks down organic matter in the absence of oxygen, producing biogas (mainly methane) and digestate, which can be used as fertilizer.
Billet Planting	A method of planting where sections of stems (billets) from established plants are used to propagate new plants, commonly employed in the cultivation of sugarcane.
Biomass	Organic material derived from living or recently living organisms, used as a renewable energy source. Biomass can include plant and animal materials.
Carbon sequestration	The process of capturing and storing atmospheric carbon dioxide to mitigate climate change. This can occur naturally in forests, soils, and oceans or be engineered by human technologies.
CEEDS TM	Crop Expansion, Encapsulation and Drilling System
DNA	Deoxyribonucleic acid, the hereditary material in all known living organisms, carrying genetic instructions crucial for development, functioning, and reproduction.
Ectophytes	Organisms, usually plants, that grow attached to the outer surfaces of other plants, often causing minimal harm or acting as epiphytes.
Endophytes	Microorganisms (often fungi or bacteria) that live within a plant without causing disease, potentially contributing to plant health and growth.
GHG	Gases that trap heat in the atmosphere, contributing to the greenhouse effect and global warming. Common GHGs include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O).
Heliotales	A grouping within the plant kingdom that includes certain flowering plants that exhibit heliotropism or are characterized by their response to sunlight, commonly discussed in botanical taxonomy.
Licensing	The process of obtaining permission from an authority or organization to carry out specific activities, such as environmental management practices or technological innovations.
Microbial	Related to microbes, which are microscopic organisms including bacteria, viruses, fungi, and protozoa, significant in various ecological and biological processes.
NEF	New Energy Farms

Nodes	Points on a plant stem where leaves, branches, or buds originate, crucial for plant growth and development.
Nutrient soaks	The practice of soaking plant materials in nutrient solutions to enhance their growth or propagation potential.
Ophiosphaerella	A genus of fungi, often studied in plant pathology, particularly in relation to its role in plant diseases.
Paludiculture	The cultivation of crops in wetlands or peatlands utilizing waterlogged conditions, often to restore ecosystems and sequester carbon.
Perennial	Plants that live for more than two years, often flowering and producing seeds multiple times throughout their lives.
Periconia macrospinosa	A species of fungus, typically involved in soil health and plant interactions, though specific details may vary.
Phragmites, Typha, Molinia & Phalaris	Genera of wetland plants commonly used in ecological projects, such as restoration and phytoremediation.
Phytoremediation	The use of plants to remove, transfer, or stabilize contaminants in soil and water, leveraging biological processes to remediate polluted environments.
Plug plants	Young plants grown in small containers (plugs) that are used for transplanting into larger spaces or landscapes.
Poaceae	The grass family, an important group of flowering plants that includes many staple crops, grasses, and sedges, crucial for various ecosystems.
Propagation	The process of growing new plants from seeds, cuttings, or other plant tissues, essential for plant reproduction.
Propagules	Any part of a plant that can reproduce to form a new plant, including seeds, spores, and vegetative parts.
REEDS	Rhizome Expansion, Enhancement & Drilling System
Saccharum	A genus of grasses including sugarcane, important for agriculture, particularly in the production of sugar and biofuels.
Spoil Land	Land that has been disturbed or altered, often due to mining or construction activities, requiring rehabilitation for productive uses.
Trichoderma	A genus of fungi known for its beneficial effects on plants, including promoting growth and disease resistance, used in agriculture and horticulture.
TRL	Technology Readiness Level
Vegetative	Referring to a mode of plant reproduction that involves asexual methods, such as cuttings or division, allowing plants to proliferate without seeds.