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Integrating perennial biomass crops into crop rotations: How to remove miscanthus and switchgrass without glyphosate

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Abstract

Perennial energy grasses have gained attention in recent years as a promising resource for the bioeconomy because of their benign environmental profile, high stress tolerance, high biomass yields and low input requirements. Currently, strong breeding efforts are being made to extend the range of commercially available miscanthus and switchgrass genotypes. In order to foster farmers' acceptance of these crops, and especially of novel hybrids, more information is required about how they can be efficiently integrated into cropping rotations, how they can be removed at the end of their productive lifespan, and what effect they have on subsequently grown crops. Farmers in Europe are meanwhile increasingly constrained in the methods available to them to remove these crops, and there is a risk that the herbicide glyphosate, which has been used in many studies to remove them, will be banned in coming years. This study looks at the removal of seven-year-old stands of miscanthus and switchgrass over 1 year at an experimental site in Southern-Germany. Three novel miscanthus genotypes were studied, alongside one variety of switchgrass, and the impact of each crop's removal on the yield of maize grown as a follow-on crop was examined. A combination of soil tillage and grass herbicides for maize cultivation was successful in controlling miscanthus regrowth, such that yields of maize grown after miscanthus did not differ significantly from yields of maize grown in monoculture rotation (18.1 t dry biomass ha^{-1}). Yields of maize grown after switchgrass (14.4 t dry biomass ha^{-1}) were significantly lower than maize in monoculture rotation caused by insufficient control of switchgrass regrowth by the applied maize herbicide. Although some regrowth of miscanthus and switchgrass was observed in the follow-on crop maize, complete eradication of both crops was achieved by subsequent winter wheat cultivation.

KEYWORDS

bioenergy, biogas, crop rotation, glyphosate, herbicide, maize, miscanthus, perennial, switchgrass

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Eva Lewin and Andreas Kiesel should be considered as joint first authors.

1 | INTRODUCTION

With the goal of mitigating their climate change impacts the European Union and their member state Germany are reducing their reliance on fossil fuels, and switching to renewable sources of energy and biomass. Recent decades have seen an increased interest in biogas as an energy source, and in bio-based substrates and products more generally. This is in part due to changes in the legal landscape, such as the implementation of the German Renewable Energy Act (Ger. EEG), which have created favorable conditions for the expansion of the biogas industry (Yang et al., 2021). Perennial biomass crops such as miscanthus and switchgrass have gained prominence in recent years because of their high biomass yield potential, low input crop cultivation, and provision of ecosystem services. These crops can increase soil organic carbon, have a low risk of nitrate leaching and erosion, and can provide habitats for wildlife (Clifton-Brown et al., 2017; Lask et al., 2020; Lewandowski et al., 2016; Mazur & Kowalczyk-Juśko, 2021; McCalmont et al., 2017).

Large efforts are being made to explore and develop potential end uses for these crops (Banerjee et al., 2022; GRACE, 2023, van der Weijde et al., 2017). Concurrently, breeding efforts are underway to improve the yields of these crops and to develop novel genotypes that are tolerant of a greater range of climatic conditions and that have properties that make them suitable for various end uses (Casler, 2021; Clifton-Brown et al., 2019). In practice, biomass from perennial grasses is still mainly used for combustion and as mulch or animal bedding. A large amount of research also focuses on developing novel bio-based, high-value material applications (e.g., GRACE project, GA Nr. 745012). Other energy conversion routes are also receiving increased attention, particularly anaerobic digestion. Studies have shown that miscanthus is able to produce biogas yields comparable to those of maize, which is currently still the dominant biogas crop in Europe, while significantly reducing the environmental impact of the substrate provision (Kiesel & Lewandowski, 2017; Kiesel, Wagner, et al., 2017; Mayer et al., 2014).

Despite these efforts, little attention has been paid to the final stage in the life cycle of perennial biomass crops, namely their removal. Effective crop removal may be necessary due to declining yields, or to allow the farmer to adjust their crop portfolio in response to market demands. An effective and efficient removal is required so that cultivation of subsequent crops can begin without economic penalty. Research on this topic is important to increase farmers' acceptance of these crops, as uptake is unlikely if the crop removal is an issue. Ordinances in Germany regulate how farmers fertilize their crops and require that crops within a rotation and their effect on the soil, such as the contribution of decaying residues, be taken into account when calculating how much fertilizer is applied (BDJ, 2017; BMEL, 2020). Thus, it is important to understand how these crops can be removed, and what effect this removal will have on the nutrient mobilization in the soil.

Studies that look at the removal of miscanthus and switchgrass are limited, mostly focusing on a single genotype and employing the herbicide glyphosate as a removal method (Anderson et al., 1997, 2011; Brown et al., 2014; Dufossé et al., 2014; Franco et al., 2018; Mangold et al., 2019; Martani et al., 2023; Rowe et al., 2020), whereby this tight focus limits how well the results of these studies can be applied more generally. The vast majority of studies on miscanthus have dealt with the standard commericial genotype *Miscanthus × giganteus* a naturally occuring hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus*. Given the large diversity within the miscanthus and switchgrass genera, it is likely that genotypes differ in how easily they can be removed, and in turn have different impacts on the soil post-removal. Similarly, it is likely that glyphosate will be banned within the European Union within coming years. It been the subject of numerous inquiries in the European union regarding its toxicity (EFSA, 2022; European Commission, 2022), and while its use is currently permitted within the European Union until the end of 2023, the German government has announced plans to phase out its usage when its current approval runs out (BMUV, 2021). For this reason, it is important to find methods of removing these crops that do not rely on glyphosate.

Recommendations on how to remove miscanthus and switchgrass suggest using a combination of glyphosate and tillage, in combination with a harvest in summertime to deplete rhizome resources (Besnard et al., 2013; Samson et al., 2016). This proved successful in the work of various authors, but this is time consuming and results in a lower yield than a harvest carried out late in the season. Dufossé et al. (2014) removed a 21-year-old stand of *Miscanthus × giganteus* through mid-season mulching of above-ground biomass, an application of glyphosate to the regrowing shoots, and deep soil tillage 4 weeks later. No significant regrowth was found in wheat grown as a follow-on crop and yields did not differ between a control and the field where miscanthus was removed. Wheat grown after miscanthus did however have fewer stems, emerged more slowly, and had a higher harvest index. The authors suggest that residual miscanthus matter led to a higher

C:N ratio and thus hindered growth in the beginning of the season. Results for switchgrass are mixed. Anderson et al. (1997) removed switchgrass through ploughing and an application of glyphosate, and did not find a negative effect on subsequent maize yields. A study by Franco et al. (2018), however, showed that yields of wheat grown following switchgrass removal were consistently lower than wheat grown in monoculture.

Mangold et al. (2019) was one of the few studies that did not use glyphosate in order to remove miscanthus. They examined the effect of removing a four-year-old *M. sinensis* stand, and fertilization on the growth of four follow-on crops: maize, ryegrass, rapeseed, and barley. Miscanthus was removed through ploughing and regrowing miscanthus shoots in follow-on crops were treated with an herbicide targeting grassy weeds. Fertilization resulted in greater suppression of miscanthus regrowth, likely due to increased vigor of the follow on crops. With the exception of rapeseed, which failed most likely due to adverse weather conditions, follow-on crops were able to successfully suppress miscanthus regrowth, either through mowing in the case of rye grass, early emergence and tillering in the case of barley, or through late soil cultivation and canopy closing in the case of maize. Despite this, low yields were observed for rye grass and barley, while maize proved a suitable candidate for cultivation following miscanthus.

The objective of this experiment is to assess the impact of miscanthus and switchgrass removal on a subsequently grown maize crop. It uses a removal method that does not involve glyphosate, as this has seldom been attempted in studies until now. The study is predicated on a scenario wherein green-harvested stands of miscanthus and switchgrass, grown for anaerobic digestion, are removed and subsequently an annual biogas crop is grown. In this experiment maize was chosen, as it had been shown to be a suitable follow-on crop. A field trial with three novel miscanthus hybrids (*M. sacchariflorus* × *M. sinensis* 'OPM16' and 'OPM17', *M. sinensis* 'OPM77'), switchgrass (*Panicum virgatum* 'Blackwell') and maize (*Zea mays* 'Kilomeris') was set up under ceteris paribus conditions for 7 years in a green-harvest regime. Removal of switchgrass and miscanthus was achieved by ploughing after the final green harvest in November 2019. In the subsequent spring, the soil was prepared and maize was sown as follow-on crop. Voluntary regrowth of miscanthus and switchgrass in the maize crop, the mineral content of the soil following removal, and the yield of the subsequent maize crop was assessed. This study brings new insight, in that it looks at a broader range of genotypes than previous studies, and employs a removal method that does not involve glyphosate.

2 | MATERIALS AND METHODS

2.1 | Experimental design

In order to test the effect of miscanthus and switchgrass removal on subsequent maize cropping, a field trial including three different miscanthus hybrids, switchgrass and maize was removed and the land resown with an annual crop, maize. The field trial was located at Ihinger Hof, a research station belonging to the University of Hohenheim in Southern-Germany. The field trial was established in 2013 as part of the EU project 'OPTIMISC' (Grant Agreement no. 289159) to assess the methane yield potential of three miscanthus hybrids and switchgrass by anaerobic digestion. Miscanthus and switchgrass were established at a planting density of 2 and 6 plants m^{-2} respectively and grown alongside maize (annually sown at 10 seeds m^{-2}) in a randomized split-block design with four replicates (plot size 5 m × 15 m) of each genotype/crop. Miscanthus and switchgrass were harvested in three different harvest regimes (double cut in July and October, early single cut in August and late single cut in October). In the original field trial (2013–2018), the miscanthus and switchgrass plots were split according to the harvest regime (split plot size 5 m × 5 m). To avoid the harvest date treatment impacting the research performed in this study, the crop was allowed two seasons to regenerate in 2018 and 2019. All miscanthus and switchgrass plots were harvested in late October each year, resulting in uniform and vigorous crops by the end of 2019 growing season. Maize cultivation was continued in the plots from the initial experiment so that maize was continually grown on these plots since 2014. In 2019 all miscanthus, switchgrass, and maize plots were fertilized the day after sowing with 150 kg N $(ha\,a)^{-1}$ given as calcium ammonium nitrate. After the final green harvest in late October 2019, the field was cultivated by ploughing on November 22nd 2019 to a depth of ~22 cm. In Table 1, the crop treatments in the initial field trial and the follow-on crop maize established after recultivation of the field are briefly characterized.

In April 2020, all plots were power-harrowed and maize 'Kilomeris' (KWS SAAT SE & Co. KGaA) was sown on April 27th 2020 at a density of 9.3 seeds m^{-2} with a row distance of 75 cm. Each plot of the recultivated field trial was sown with six rows of maize, resulting in 4.5 m × 15 m maize plots. The crop protection strategy included two herbicide applications: The first treatment was carried out 1 week after sowing by application of a tank-mixture of soil active herbicides pendimethalin (1365 g ha^{-1}, Stomp Aqua®; BASF SE) and Dimethenamid-P (900 g ha^{-1}, Spektrum®; BASF SE). Both soil active herbicides are a standard treatment in maize cultivation and are known to also be suitable for miscanthus cultivation. For this reason, volunteer miscanthus and switchgrass regrowth appeared and needed to be suppressed with a second application of a leaf-active herbicide on May 26th 2020 containing 63 g ha^{-1} foramsulfuron, 2 g ha^{-1} iodosulfuron, 20 g ha^{-1} thiencarbazone, and 30 g ha^{-1} cyprosulfamide (MaisTer® power; Bayer Crop Science Deutschland GmbH). For fertilization, 198 kg N ha^{-1} was applied as stabilized urea including dicyandiamid und 1H-1,2,4 triazol as a nitrification inhibitor (Alzon 46; SKW Stickstoffwerke Piesteritz GmbH) in one application directly after sowing.

TABLE 1 Maize, miscanthus, and switchgrass genotypes used in the previous experiment and during recultivation.

Field trial duration	Treatments				
2013–2019	Maize 'Kilomeris' (*Zea mays* 'Kilomeris') annually re-sown each spring, harvest in September, soil cultivation in winter, four replicates	Miscanthus 'OPM 16', perennial *Miscanthus sacchariflorus* × *Miscanthus sinensis* hybrid with a non-creeping rhizome and a compact bunch of shoots (plant diameter ~50 cm), flowering in mid-August. *Source*: Aberystwyth University, four replicates	Miscanthus 'OPM 17', perennial *M. sacchariflorus* × *M. sinensis* hybrid with a creeping rhizome typical for *sacchariflorus*; flowering in mid-August and active, early senescence starting from early September. Source: Aberystwyth University, four replicates	Miscanthus 'OPM 77', perennial *M. sinensis* hybrid with a compact bunch of shoots typical for *M. sinensis* (plant diameter ~40 cm); flowering in late August. *Source*: Wageningen University, four replicates	Switchgrass 'Blackwell' (*Panicum virgatum* 'Blackwell'). Perennial upland ecotype with a very compact bunch of shoots (plant diameter ~20 cm); flowering in mid-July, four replicates
2020	Maize 'Kilomeris' (*Zea mays* 'Kilomeris'), sowing density at 9.3 seeds m^{-2}, chemical crop protection				

2.2 | Weather data

Weather data were collected from a weather station located approximately 500 m from the test site operated by the Agricultural Technology Centre (LTZ, 2023). Temperature measurements were taken 2 m above the soil surface. Monthly averages of temperature and precipitation totals for each month were sourced from the LTZ as well as long term data from 1981 to 2020, which was used as a long-term baseline comparison. Water balance data were also calculated by the LTZ. The average temperature at Ihinger Hof for 2020 was 10.2°C with total precipitation of 585 mm (Figure 1). This shows that the year 2020 was considerably warmer and drier than the long-term average for Ihinger Hof from 1981 to 2010 (annual average temperature 8.60°C, average annual precipitation 704.2 mm).

2.3 | Physical measurements of crop growth

To assess the development of the maize crop, plant measurements were taken during the vegetation period on May 15th 2020, June 5th 2020 and August 6th 2020. The measurements included number of maize plants m^{-2}, volunteer miscanthus and switchgrass shoots m^{-2}, and average canopy height of the maize crop and volunteer miscanthus and switchgrass regrowth. The number of maize plants and miscanthus and switchgrass shoots was measured in the center of the maize plot over an area of ~1 m^2 (0.75 m × 1.33 m). The average canopy height of maize and volunteer miscanthus and switchgrass was estimated by using a measuring stick and was measured from the ground to the highest fully developed leaf. During these plant measurements the plots were also checked for volunteer growth of other weeds (i.e., neither miscanthus nor switchgrass) but due to the effective chemical weed management no significant quantity of weeds was found.

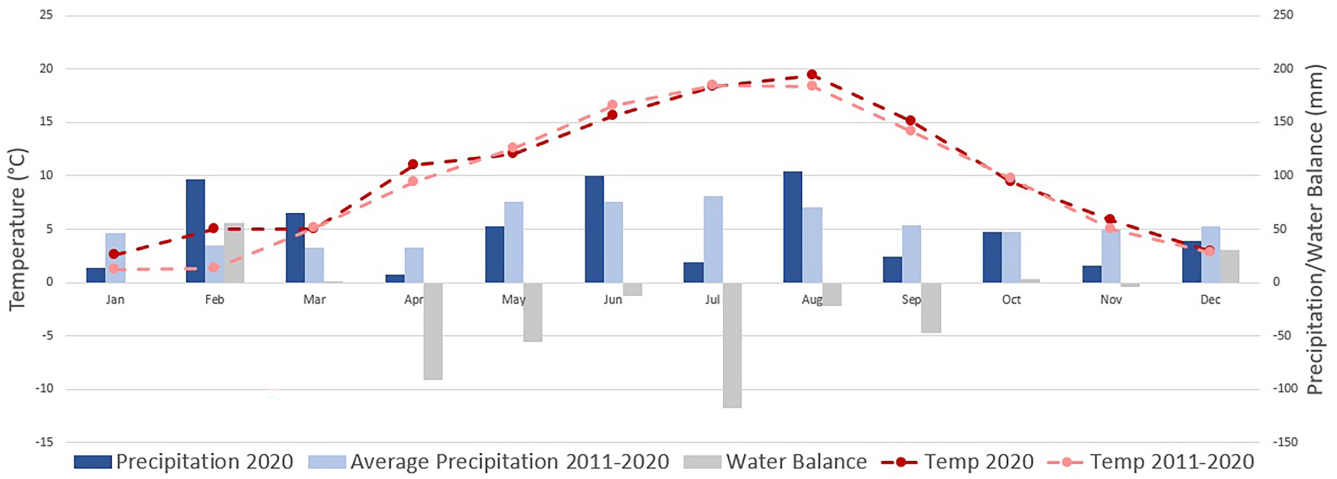

FIGURE 1 Weather data from Ihinger Hof for 2020, and long term weather data at the site from 1981 to 2010. For review only: Dark red and blue represent measurements from 2020. Light blue and light represent long term averages. The monthly water deficit calculated based on evaporation and precipitation is shown in grey.

2.4 | Yield and mineral offtake data collection

Two methods were used to assess the yield of maize and regrowing miscanthus and switchgrass.

Total harvestable fresh biomass yield was estimated on September 17th 2020 using a BAURAL SF 2000 (Zürn Harvesting GmbH & Co. KG) self-propelled plot forage harvester. The third and fourth maize rows, in the center of the plot, were harvested at a cutting height of ~20 cm and along 13.6 m stretch resulting in a sampling area of 20.4 m^2. The harvested biomass was weighed during harvest by the harvester to assess the fresh biomass yield and a sample was taken for dry matter content analysis. Volunteer miscanthus and switchgrass biomass was not removed prior to harvesting with the forage harvester but due to poor regrowth, their contribution to the overall yield was only minor. Yield calculated from this harvest was called harvestable yield, and reflected yields as they would be achieved in praxis.

A second yield determination was carried out by harvesting maize plants and regrowth by hand. A 1 m^2 area (0.75 m × 1.33 m) was harvested manually in the fifth maize row at a cutting height of ~5 cm. During the hand harvest, maize and volunteer miscanthus and switchgrass biomass was collected separately and the fresh weight of the biomass samples was assessed by weighing on a scale. After weighing, the fresh biomass was chipped using a laboratory chipper and a representative sample was taken for further chemical analysis. This was called potential yield as it represented the amount of biomass that could potentially be taken from the field. Potential yield was calculated for maize and for switchgrass and miscanthus regrowth.

In both harvests, biomass samples were dried at 80°C to a constant weight in a drying cupboard. The dry weight was then used to calculate the dry matter yield. Winter wheat was sown following the maize harvest, though data about the yield and growth of this crop were not collected.

2.5 | Chemical analysis of harvested biomass

The dried subsample of pure maize, miscanthus, or switchgrass was then milled in a cutting mill SM 200 (Retsch) using a 1-mm sieve. The milled subsample was then used for analysis of ash, nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) content. The nitrogen content was analyzed using a Vario Macro Cube (Elementar Analysensysteme GmbH) in which the samples are completely incinerated with oxygen and content of nitrogen compounds in the resulting off-gas was measured by a thermal conductivity detector. The P, K, Mg, and Ca contents were measured by microwave digestion of the biomass, followed by analysis of the extract using inductively coupled plasma optical emission spectroscopy (ICP-OES). For this analysis, 0.5 g of each sample was diluted with 8 mL HNO_3 and 6 mL H_2O and digested in an ETHOS.lab microwave (MLS GmbH). The ICP-OES analysis of the extract was performed by Core Facility Hohenheim, which is the central laboratory of the University of Hohenheim.

2.6 | Soil sampling

Soil samples were taken on four dates to examine the nutrient dynamics in the soil during the vegetation periods. On the first date, April 15th, multiple samples were taken from the field and combined to use as a baseline for further calculations. Samples were then taken on May 18th, June 24th, and after the harvest on September 22nd 2020. Soil samples were taken with an auger to a depth of 90 cm and divided into three fractions (0–30 cm, 30–60 cm, 60–90 cm), which were then analyzed separately. Directly after extraction from the soil, the soil samples were cooled to avoid ammonium losses and stored frozen until analysis. Following extraction stones and other debris were removed from soil samples.

Plant available nitrogen in fresh soil (NO_3 and NH_4; referred to as N_{min}) was determined using a $CaCl_2$ extraction and FIA (flow-injection analysis) measurement (DIN ISO 14255:1998-11). The amount of plant available phosphorus and potassium was then analyzed in dried soil via CAL-extraction followed by measurement with a flame photometer or FIA respectively (OENORM L 1087:2012-12-01). Soil pH was determined with a glass electrode after $CaCl_2$ extraction (DIN ISO 10390:2005).

2.7 | Nitrogen balance in the soil

Soil samples taken from the beginning of the vegetation period until after maize was harvested were used to calculate a nitrogen balance. A mixed probe was used for the first value; hence, for all treatments initial soil nitrogen is given as 18.15 kg ha^{-1}. Mineral offtake calculated from harvest data was compared with measured soil nitrogen.

2.8 | Statistical analysis

Statistical analysis was conducted in R (R Core Team, 2020) and RStudio (Version 2022.02.3). The program 'agricolae' was used to perform an ANOVA and Tukey's test using the following model.

$$y = \mu + \text{prev} + B + e,$$

where y=yield of maize or regrowth of miscanthus or switchgrass, μ=general mean effect, prev=effect of the previous crop, B=effect of block, and e=residual error.

Homogeneity of variance was assessed visually. The effects were tested at a level of probability of $\alpha = 0.05$.

3 | RESULTS

3.1 | Regrowth of miscanthus and switchgrass under maize

Maize emergence and development in early summer is shown in Figure 2. Figure 2a1,a2 show the development of maize grown after maize and it is visible that the first round of herbicide treatment was sufficient to control any weed growth. In the maize crop following miscanthus (Figure 2b–d) and switchgrass (Figure 2e), volunteer growth of the previous crop is visible, mostly in OPM17 (Figure 2c1,c2) and switchgrass (Figure 2e1,e2), and least of all in OPM77 (Figure 2d1,d2). In OPM16 (Figure 2b1,b2), volunteer growth of miscanthus was mainly found in clumps, while volunteer growth in previous OPM17 and switchgrass treatments was found almost uniformly distributed across the whole area. The middle row of photos was taken 10 days after application of the grass herbicide and leaf yellowing and a small amount of anthocyanin pigmentation could be observed in miscanthus and switchgrass. Although the plants still appeared green at this time, the effect of the herbicide was visible. This application was important to allow the follow-on crop maize to outcompete the volunteer miscanthus and switchgrass growth.

3.2 | Physical measurements of crop growth

At the end of the vegetation period maize height (Figure 3, black circles) was greatest in maize grown after maize (277 cm) and lowest in maize grown after switchgrass (246 cm), whereby closer analysis is difficult due to the low number of measurements. Regrowing miscanthus and switchgrass plants were smaller than maize. Regrowing switchgrass (72 cm) was taller than all miscanthus genotypes and OPM77 (66.25 cm) was taller than both OPM17 (47.5 cm) and OPM16 (56.25 cm). Switchgrass produced a greater number of stems (113.75) than all three miscanthus genotypes and continued to produce stems throughout the growing season. Miscanthus produced fewer stems than switchgrass and had less stems at the end of the growing season following the application of herbicides in May. OPM77 had fewer stems (21) than OPM16 (43) and OPM17 (35) on the last measurement date, and throughout the whole vegetation period.

3.3 | Yield of maize and regrowing miscanthus and switchgrass

The potential maize yield averaged 21.94 t dry mass (DM) ha^{-1} (Figure 4A) across all treatments and was higher than the average harvestable maize yield 16.52 t DM ha^{-1} (Figure 4B). In plots where miscanthus or switchgrass was grown previously, the volunteer regrowth of these crops averaged 0.33 and 1.18 t DM ha^{-1}, respectively (Figure 4A).

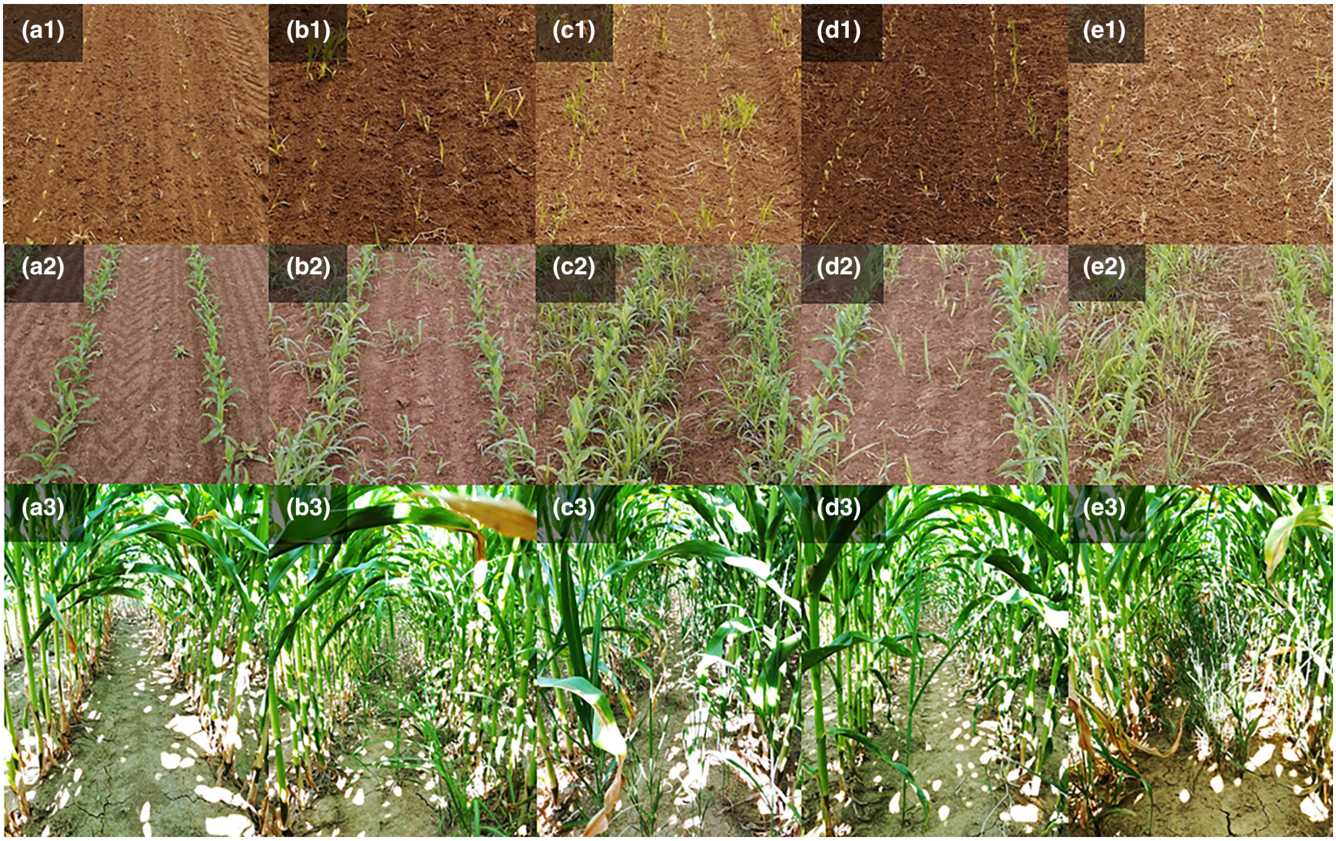

FIGURE 2 Growth of maize and volunteer regrowth of miscanthus and switchgrass on May 15th 2020 (a1–e1), June 5th 2020 (a2–e2), and August 6th 2020 (a3–e3). From left to right: (a) Maize grown after maize, (b) maize grown after OPM16, (c) maize grown after OPM17, (d) maize grown after OPM77, and (e) maize grown after switchgrass.

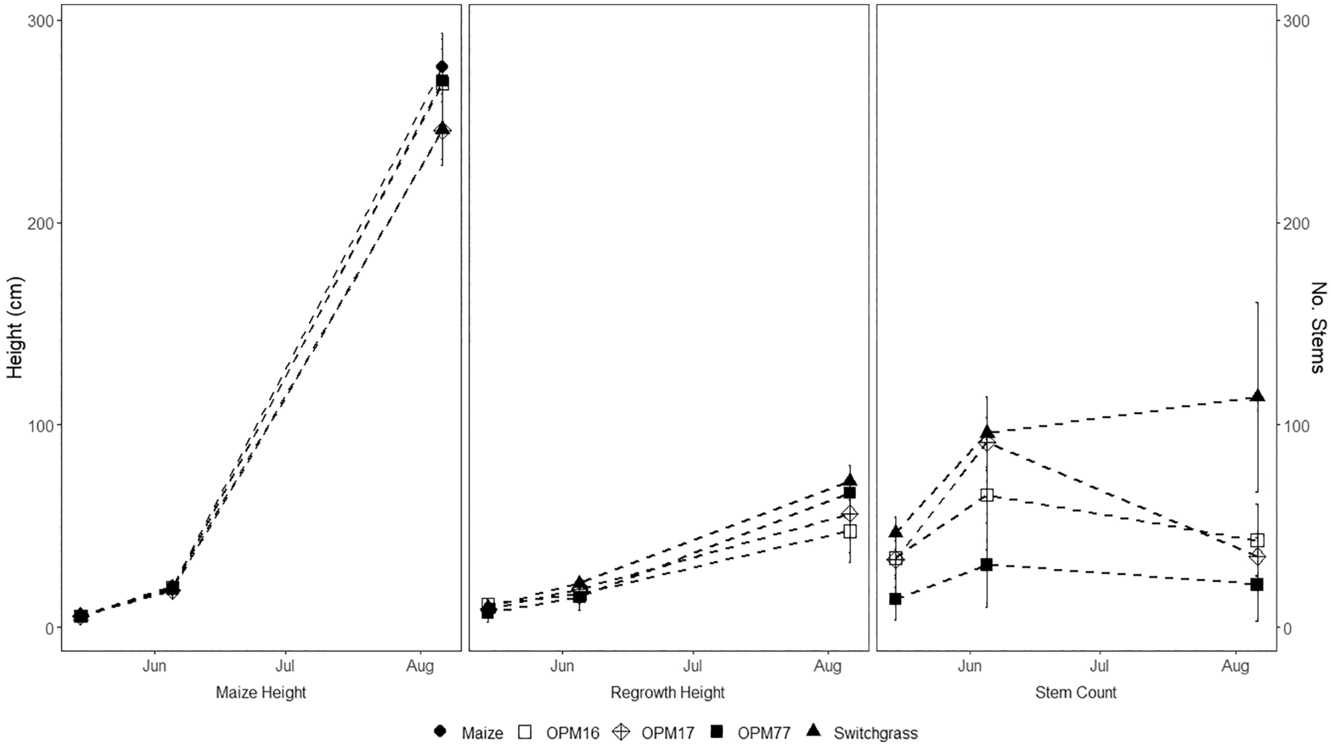

FIGURE 3 Field measurements of maize and miscanthus and switchgrass regrowth in 2020 following removal. $N=4$ per treatment.

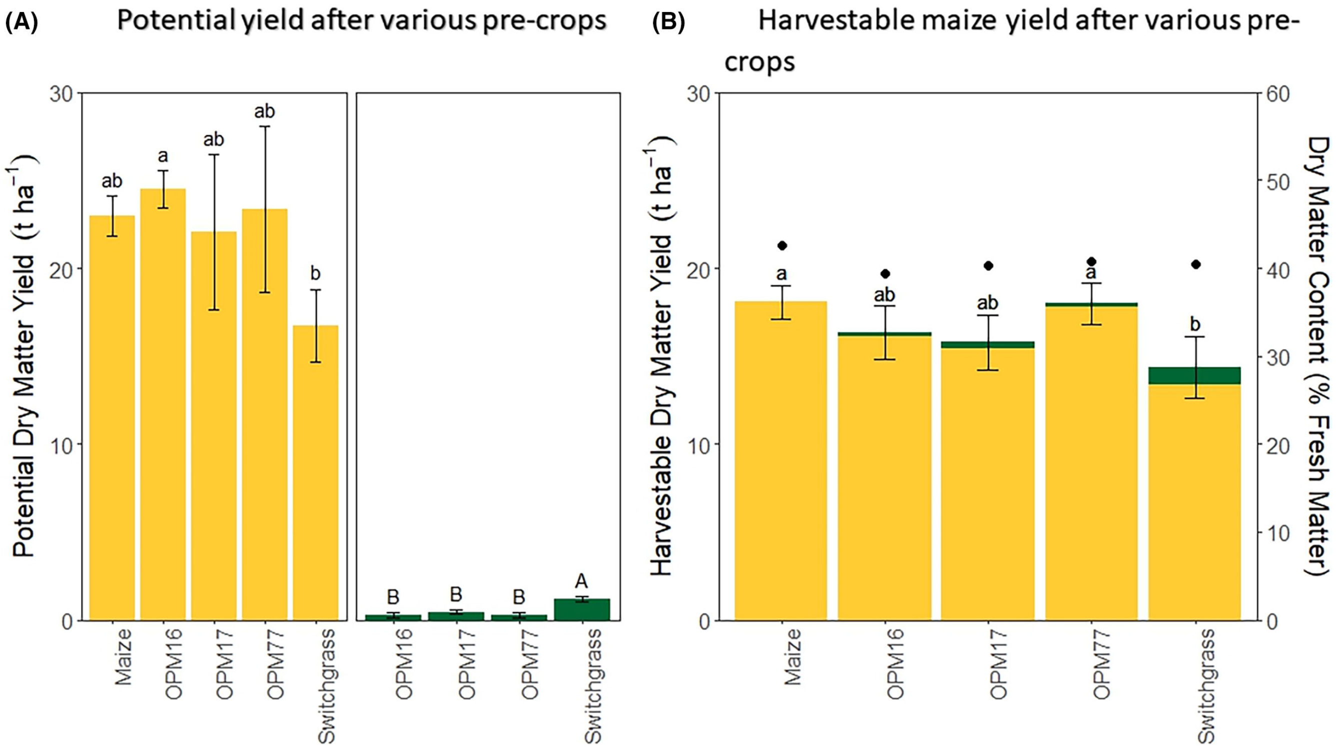

FIGURE 4 (A) Potential dry matter yield of maize after various pre-crops (maize, miscanthus (OPM16, OPM17, OPM77) and switchgrass) (yellow) and potential regrowth yield of miscanthus and switchgrass (green), (B) harvestable dry matter yield. The forage harvester used to determine harvestable yield harvested all plant material together, and did not separate maize from miscanthus and switchgrass regrowth. Hand harvest data was used to estimate the fraction of the harvestable yield made up by maize and regrowth respectively. Yellow represents the fraction made up by maize, and green represents the fraction made up by regrowing miscanthus or switchgrass. Black dots represent the dry matter content of harvested biomass a percentage of total fresh matter. Error bars represent standard deviation. Columns with different letters differ significantly at $\alpha = 0.05$. $N = 4$ per treatment.

Harvestable yield contained a mixture of maize plants and switchgrass or miscanthus regrowth. For this reason, the data from the potential yield measurement were used to estimate the proportion of maize and regrowth in the harvested material (Figure 4B).

Potential yield was calculated based on hand-harvest data. Maize grown after OPM16 showed the highest potential yield (24.5 t DM ha^{-1}), and yielded significantly higher than maize grown after switchgrass (16.8 t DM ha^{-1}) (Figure 4A). No significant differences were detected in the regrowth yield of different miscanthus genotypes; however, switchgrass produced significantly more regrowth than all miscanthus genotypes.

The harvestable yield of maize grown after maize did not differ significantly from any of the miscanthus treatments (Figure 4B). No significant differences were observed between the harvestable yields of different miscanthus treatments. The harvestable yield of maize grown after switchgrass was significantly lower than maize grown after maize and maize grown after OPM77. The harvestable yield of maize grown after switchgrass did not differ from maize grown after OPM16 or OPM17. Dry matter content was highest in maize grown after maize but did not differ greatly from other treatments.

3.4 | Biomass mineral content and offtake

No significant differences were observed for K, Ca, or Mg content in harvestable maize biomass (Figure 4A). P content was highest in maize grown after switchgrass, and this was significantly higher than the P content in maize grown after maize and maize grown after OPM16. N content was highest in maize grown after OPM77, significantly higher than maize grown after maize and maize grown after OPM16. OPM16 regrowth contained significantly more Ca than other genotypes (Figure 5B). Switchgrass regrowth contained significantly more Mg than other genotype and the highest K content which was significantly higher than that of OPM77, which had the lowest K content. OPM77 regrowth had the highest P content with significantly more than OPM17 and switchgrass regrowth. OPM17 regrowth had the least Mg

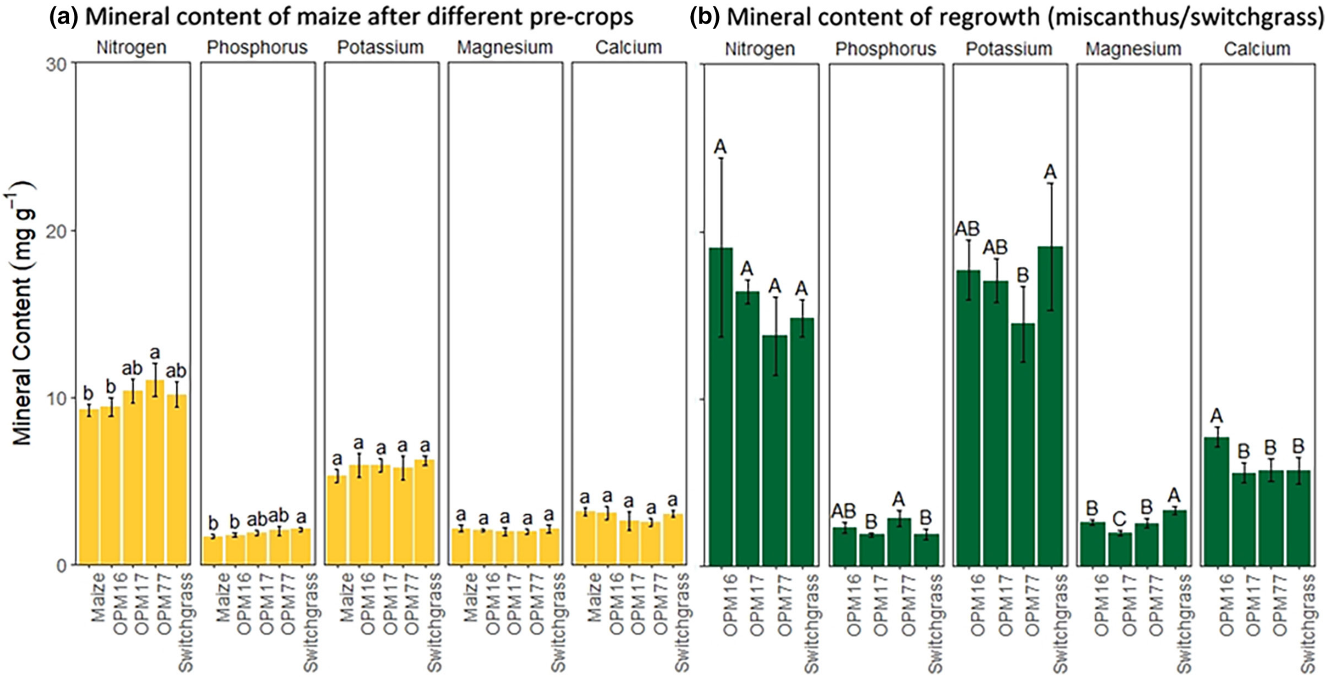

FIGURE 5 (a) Mineral content of harvestable biomass (b) mineral content of hand-harvested miscanthus/switchgrass regrowth. Error bars represent standard deviation. Columns with different letters differ significantly at $\alpha = 0.05$, $N = 4$ per treatment.

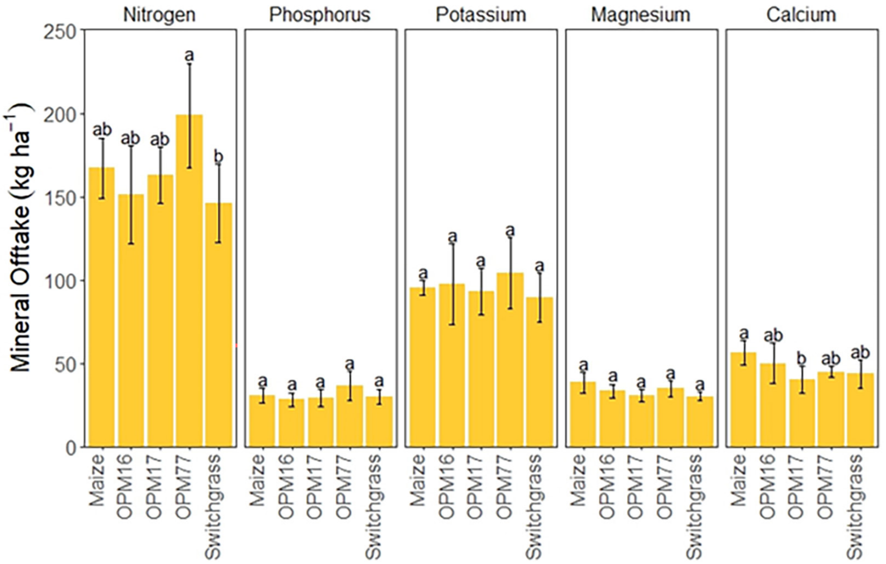

FIGURE 6 Mineral offtake in harvestable biomass. Error bars represent standard deviation. Columns with different letters differ significantly at $\alpha = 0.05$, $N = 4$ per treatment.

and low P compared with other genotypes. There were no significant differences detected in the N content of regrowth.

Mineral content data were combined with calculated harvestable dry matter yields to estimate mineral offtake. No significant differences were observed between treatments for the offtake of P, K, or Mg. N offtake in maize grown after OPM77 was the highest and was significantly higher than maize grown after switchgrass. Ca offtake was greatest in maize grown after maize and lowest in maize grown after OPM77, whereby the only significant difference observed was between these two treatments.

3.5 | Dynamics of soil mineral content

Differences in soil K and P were relatively minor (data not shown). Soil samples showed relatively stable levels of K and P in the soil layers 30–60 and 60–90 cm. Mid-season levels of K were much higher in maize after maize and maize after OPM16 than in other rotations. P levels increased towards the middle of the season before remaining constant or declining slightly towards the end of the season. In maize following OPM16 P levels decreased through the entire growing season. Few differences in N_{min} were found in the 30–60 or 60–90 cm layers. Figure 6 shows the N_{min} levels of various treatments in the 0–30 cm fraction. N_{min} levels increased in all treatments towards the middle of the growing season due to an April application of fertilizer before declining in all treatments. N_{min} levels were similar in maize after maize, maize after OPM16 and maize after OP77 at the end of the season. The treatment maize after OPM16 had much higher soil N_{min} at the end of the season in 0–30 cm and maize after switchgrass much lower N_{min}.

N_{min} increased in all treatments except for switchgrass where the end value was slightly lower than the initial value. An estimated final soil N_{min} value was calculated by combining the initial soil N_{min} concentration with fertilization and offtake data, and this was then compared with the measured final value. N_{min} at the end of the growing season was similar to the estimate for maize grown after maize and OPM17 and considerably higher for maize grown following OPM16 and OPM77. The final measured N_{min} concentration in maize grown after switchgrass was much lower than the estimated value.

4 | DISCUSSION

The objective of this study was to examine how novel miscanthus and switchgrass genotypes can be removed and how successfully these fields can be recultivated with maize. Ultimately, the results show that miscanthus can be removed effectively through a combination of soil tillage and standard maize herbicide treatment without the use of glyphosate. Switchgrass removal proved more challenging, and a decrease in maize yield was observed following removal, suggesting a different herbicide strategy is necessary.

Although complete eradication of miscanthus was not achieved during the cultivation of the follow-on crop maize, no significant difference was observed between the yield of maize grown after miscanthus and maize in a monoculture rotation. After the final maize harvest, the soil was cultivated and winter wheat was sown in October. Neither miscanthus nor switchgrass showed any regrowth in the subsequent June (data not shown), which proved complete removal of both perennial crops within one vegetation period. Soil sampling and the mineral content of harvested maize suggests that the hybrids OPM77 and OPM16 led to soil mineralization while switchgrass likely caused immobilization in the soil.

The following sections discuss the yield of maize following removal, the efficiency of the removal, the effect of removal on the soil, other factors affecting removal, and recommendations for farmers.

4.1 | Maize yield following removal

Maize yield was assessed through a hand harvest (potential yield, Figure 4A) and harvest with a forage harvester (harvestable yield, Figure 4B). The average potential yield of maize was 21.94 t ha^{-1}, which was higher than the average harvestable yield of 16.52 t ha^{-1}, whereby the variance in the potential yield was much greater. This discrepancy may result from the lower cutting height in the hand harvest (5 cm) compared to the forage harvest (20 cm). Densley et al. (2001) observed a yield loss of 1 t ha^{-1} for silage maize when cutting height was increased from 10 to 30 cm while Magenau et al. (2021) demonstrated yield losses of 0.27 t ha^{-1} for each 1 cm increase in cutting height for harvests of miscanthus. The value found by Densley et al. (2001) suggests that cutting height alone is not responsible for the discrepancy. Another explanation may be that the potential yield value was inflated as it was gathered from a much smaller harvest area.

In both harvests, maize grown after switchgrass produced the lowest amount of biomass. The harvestable yield of maize grown after switchgrass was significantly lower than the yield of maize grown after maize and maize grown after OPM77. Numerous factors can explain the low yield in this treatment. Firstly, regrowing switchgrass was not adequately suppressed by the application of herbicide in May. Field measurements show that switchgrass recovered better than miscanthus following the application of grass herbicide in May, reaching a higher stem number and height at the harvest than before herbicide application (Figure 3). Miscanthus on the other hand recovered more slowly and produced fewer new shoots after the application (Figure 3). At the end of the growing season switchgrass had produced significantly more biomass than any of the miscanthus genotypes examined, but it cannot be excluded that another herbicide or a second application of a grass herbicide could have minimized switchgrass regrowth (Figure 4A).

Secondly, weather conditions were abnormally dry in 2020. Almost no precipitation fell in April (4.8 mm) at this location leading to relatively dry soils very early in the year. After maize sowing in late April, the soils were re-wet by precipitation in May and June but the water balance was still negative for both months (−55.6 mm, and −12.3 mm respectively), showing that the precipitation was not sufficient to offset the soil water deficit that had accrued since the spring. From June 20th onwards, almost no significant precipitation occurred until a heavy rain fell on August 17th (when more than 40 mm fell), resulting in a distinct drought period in early summer. Under such conditions, competition for water between regrowing volunteer crops and maize was high, especially at the beginning of the cultivation period before the herbicide treatment, when regrowing miscanthus and switchgrass showed high stem counts (Figure 3).

Thirdly, immobilization may have occurred in the soil caused by decaying rhizome biomass from the previous switchgrass crop. Soil sampling of maize grown after switchgrass suggests that immobilization had occurred in this treatment, resulting in lower available N_{min} compared to other treatments. However, the N content of maize grown after switchgrass was not significantly different from other treatments, suggesting that N_{min} was sufficient. Anderson et al. (1997) found no negative effect of switchgrass removal on the yield of subsequent maize, but recorded no significant switchgrass regrowth when maize was sown. In a similar experiment Franco et al. (2018) showed that yields of wheat following switchgrass were lower than wheat grown in monoculture, which they attributed to immobilization. It seems plausible that the low yield of maize grown after switchgrass in this experiment was caused by a combination of the factors stated above. Switchgrass competed with maize for water and nutrients in the early season, and mineral nitrogen was less available than in other treatments due to immobilization.

The harvestable dry matter yield of maize grown after OPM77 (18.00 t DM ha^{-1}) was in the range of maize grown after maize (18.09 t DM ha^{-1}). This may be due to the small amount of regrowth produced by this genotype, or as a result of higher N availability following mineralization of miscanthus biomass (Figure 6). As OPM77 and OPM16 produced similar amounts of regrowth (Figure 3a) it is likely that the higher yield of maize following OPM77 compared to OPM16 is due to higher N availability in this treatment due to mineralization. This is supported by soil samples (Figure 7) that show greater mineral N availability in the OPM77 treatment than in the OPM16 treatment in the middle of the vegetation period. It is also supported by the high N content and offtake seen in harvested maize biomass from this treatment (Figures 5 and 6). Maize grown after OPM77 contained significantly more N than maize grown after maize or OPM16. The N offtake in maize grown after OPM77 was also much higher than other treatments and significantly higher than the offtake in maize grown after switchgrass.

Excluding N, differences in the mineral content of harvested maize were generally minor.

Mangold et al. (2019) used a hand harvest method to assess the yield of maize following miscanthus removal and reported a yield of 26.92 ± 1.17 t dry matter ha^{-1} for fertilized maize. This corresponds well to the potential yield in this experiment (21.94 t ha^{-1}), considering the extended drought conditions in this study. This experiment and that of Mangold et al. (2019) took place at the same experimental station and used the same protocols to estimate maize yield, however mangold et all applied 240 kg ha^{-1} N, more than was applied in this experiment. Another experiment at this site by Xu et al. (2017) observed long-term average maize yields of 18.5 t dry matter ha^{-1} when grown in monoculture and fertilized with

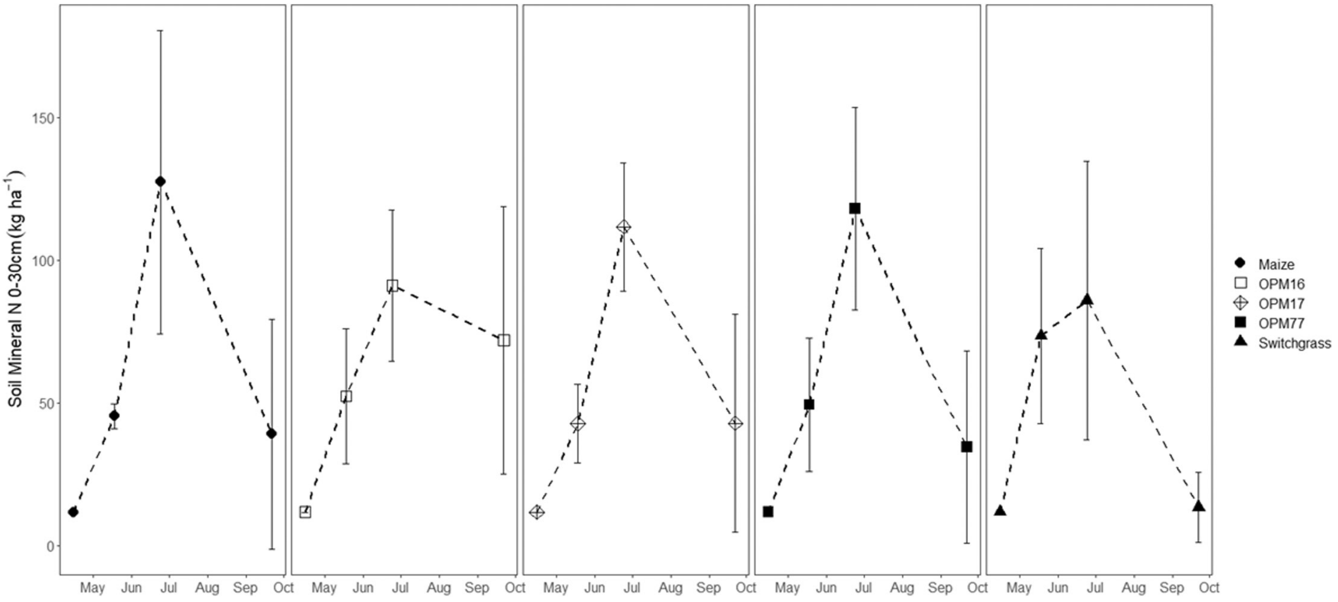

FIGURE 7 Mineral content of soil fractions at three dates in 2020, $N = 4$.

240 kg ha^{-1} nitrogen. Maize in the experiment by Xu et al. (2017) was harvested at a cutting height of 20 cm and should thus be compared to the harvestable maize yield of this experiment which was 16.52 t ha^{-1}. The difference can again be attributed to the dry conditions in summer. Thus, it can be said that the maize yield in this experiment corresponds well to the results attained for monoculture maize at this site.

4.2 | Removal of miscanthus and switchgrass

This experiment built upon the work of Mangold et al. (2019) and employed a wider variety of genotypes as well as switchgrass. The protocol in this experiment differed in a few small points: Miscanthus was harvested in October as opposed to February, and for the removal, step soil cultivation was used in combination with the application of an herbicide containing foramsulfuron, iodosulfuron, thiencarbazone, and cyprosulfamide. Mangold et al.'s experiment also did not contain a control of maize in monoculture. Miscanthus was adequately suppressed such that no significant difference between the harvestable yield of maize after maize and maize after miscanthus was detected. Average miscanthus regrowth (326 ± 160 kg DM ha^{-1}) (Figure 4A) was also similar to the value achieved by Mangold et al. (2019) of (360 ± 310 kg DM ha^{-1}) for fertilized maize. Mangold et al. (2019) experiment, showed that maize, barley, and ryegrass were all suitable options for competitive follow-on crops for miscanthus removal, though yields were low in barley and ryegrass and hence maize was chosen for this experiment.

The results of this study show that soil cultivation, in this case ploughing in November followed by seedbed preparation using a power-harrow just before maize sowing in May was sufficient to reduce the regrowth of miscanthus and switchgrass allowing maize to be sown without any technical issues. However, since miscanthus and switchgrass rhizomes were only damaged and not completely destroyed by soil cultivation, volunteer miscanthus and switchgrass regrowth occurred, which required control by application of a leaf-active grass herbicide to minimize competition with maize until suppression of weeds by maize after the 6-leaf stage. The regrowth was dependent on the hybrid and least regrowth was observed in OPM77, an *M. sinensis* species, which are known to produce smaller rhizomes. Overall, the combination of soil tillage and herbicide application provided a good control of miscanthus and some degree control of switchgrass regrowth, though it did not completely eradicate both, and allowed maize cultivation directly after removal of the perennial crop. This is very important for farmers' acceptance of perennial crops, as this allows cultivation of the next crop in direct succession. After whole plant maize harvest for ensiling in September, the soil was again cultivated and winter wheat was sown. In the following spring, the field was checked for miscanthus and switchgrass regrowth in June but no regrowth was observed, which was considered as complete removal of miscanthus and switchgrass.

Other studies have also applied a combination of tillage and herbicide application to remove miscanthus. Dufossé et al. (2014) removed a 20 year old stand of *Miscanthus × giganteus* in accordance with guidelines from Besnard et al. (2013), using glyphosate and tillage. Total removal of miscanthus was achieved in plots recultivated with wheat, and regrowth of <1 t DM ha^{-1} was observed on plots left fallow after removal. Similarly, Anderson et al. (2011) tested removal of *Miscanthus × giganteus* using various combinations of tillage and glyphosate applications in the USA, and found that of the combination of tillage glyphosate application provided the best removal success for *Miscanthus × giganteus*. The results of this experiment present evidence that a combination of herbicide and tillage works to remove miscanthus hybrids of *M. sinensis* and *M. sacchariflorus*, even in a maize follow-on crop. Moreover, this study showed that especially *M. sinensis* was very easy to remove and might not even require an additional herbicide application, most probably as it produces less rhizome biomass.

Previous studies showed that application of glyphosate, especially in combination with soil tillage, is effective for removal of miscanthus and switchgrass, and to control regrowth (Anderson et al., 1997, 2011; Brown et al., 2014; Dufossé et al., 2014). However, glyphosate application is controversial due to the potential health and environmental risks associated with its use. Political debate about the permissibility of glyphosate is ongoing on the EU and the national level (EFSA, 2022; European Commission, 2022). In this unclear legislative situation, alternative herbicides and measures to remove perennial crops are very important for the farmer and the acceptance of novel cropping systems such as miscanthus. In this study, we were able to show that miscanthus and switchgrass can be effectively removed without the use of glyphosate, with control of regrowth after soil cultivation achieved with a conventional leaf-active maize herbicide based on a range of sulfonylurea active ingredients. This approach is advantageous compared to glyphosate application as maize sowing can be carried out at the ideal sowing time in May. A successful glyphosate application requires that weeds being targeted reach a certain developmental stage before spraying can occur, which would then result in a delayed maize sowing as maize is susceptible to glyphosate. This would not be the case for some transgenic varieties of maize; however, these are not commonly cultivated in the European Union.

This experiment differed from others in that the period of time between removal and maize sowing was greater. In the case of Dufossé et al. (2014) and Mangold et al. (2019), cultivation of the follow-on crops began shortly after miscanthus removal, whereby in both experiments an immobilization effect was observed. In the case of Dufossé et al. (2014) the immobilization effect was visible as a change in harvest index and stem no. of wheat, whereby in the study by Mangold et al. (2019) observed yields of rye grass and barley were lower than expected, while maize yields were high. This is likely because not enough time had passed between removal and planting of a follow-on, to allow the underground miscanthus biomass to degrade sufficiently. It is however unclear why this phenomenon was only observed in some of the crops examined. A green harvest followed by spring sowing of maize, as used here, could be recommended, as it allows more time for the degradation of biomass and leaves a greater window for soil preparation before the follow-on crop is sown. Miscanthus is damaged by the initial removal through ploughing, and again by the seedbed preparation just before sowing of the maize. Guidelines for miscanthus removal recommend harvesting miscanthus early in order to deplete nutrient reserves in the rhizomes (Besnard et al., 2013; Samson et al., 2016); however, the results of our study show that miscanthus can be allowed to ripen until the end of the growing season, without a detrimental effect on the removability. This results in a greater miscanthus yield than an early harvest in June would provide (Kiesel, Nunn, et al., 2017; Magenau et al., 2022).

The herbicide applied against grass species in this experiment is a typical post-emergence herbicide for maize cultivation and contains foramsulfuron, iodosulfuron, thiencarbazone, and cyprosulfamide as active ingredients. The class of sulfonylurea herbicides is known to damage grass species including miscanthus and switchgrass, although single representatives can even be used to control weeds in miscanthus (e.g., Rimsulfuron) and switchgrass (Nicosulfuron). Everman et al. (2011) demonstrated that *M. sinensis* and *Miscanthus*×*giganteus* show different degrees of tolerance to this class of compounds. For example, Nicosulfuron has been shown to be effective at controlling *M. sacchariflorus* and *Miscanthus*×*giganteus*, and less effective at controlling *M. sinensis* (Everman et al., 2011; Song et al., 2016). While nicosulfuron was not used in this experiment, it suggests that differences in herbicide tolerance between genotypes will arise as breeding of new genotypes progresses. However, as *M. sinensis* produces less rhizome mass leading to less regrowth after soil cultivation, the control of *M. sinensis* regrowth seems less challenging and a higher tolerance for herbicides such as nicosulfuron not problematic. In this study, also the *M. sinensis* regrowth was controlled very well by the applied sulfonylurea herbicide and the follow-on crop maize even performed best following the *M. sinensis* hybrid OPM77, while OPM16 and 17 led to slightly lower, but not significantly different maize yields compared to the control maize after maize. Conversely, a significant yield reduction of maize following switchgrass compared to maize following maize was observed, which shows the reduced ability to control volunteer switchgrass regrowth by the applied herbicide. Kering et al. (2013) showed that foramsulfuron damages switchgrass, but measured visual damage of 16% at 30 days following application, suggesting that it could be even used to control weeds in switchgrass. This experiment used a higher concentration of foramsulfuron than Kering et al. (2013), though as previously stated, switchgrass recovered over time. Despite the observed yield reduction in this experiment, the control of switchgrass can be still considered as sufficient, considering the heavy drought conditions, the resulting water competition in spring before the herbicide application, and the total eradication of switchgrass by winter wheat in the following season.

4.3 | Impact of miscanthus removal on soil mineral nitrogen

Mineral nitrogen (N_{min}) amount in soil increased in all treatments except switchgrass over the course of the 2020 vegetation period (Table 2), in part due to fertilization but likely also due to the mineralization of N from soil and degradation of biomass. Mineral offtake was lower than fertilization for all treatments except maize following OPM77 where the fertilizer applied was equal to the N removed through maize biomass. This suggests that the applied fertilizer demand was overestimated for maize following maize, OPM16, OPM17, and switchgrass, which is most likely a result of the abnormally dry conditions in 2020 that have lowered the yield and thus the nitrogen uptake. Average N_{min} in soil was 47.33 kg N ha^{-1} at the last sampling date. High soil N_{min} at the end of the season is undesirable as it can be leached from the soil over winter but unproblematic in this trial, since winter wheat was sown after maize harvest which is able to take up mineral nitrogen from the soil in autumn.

An N balance was calculated using soil samples taken at the beginning and end of the vegetation period and offtake data from the forage maize harvest (Table 3). In maize grown after maize the amount of N_{min} in

TABLE 2 *p* Values for *F* tests of the fixed effect of previous crop in rotation for yield, mineral content, and mineral offtake.

	***F* value of "previous crop"**	***p* (>*F*)**
Harvestable yield	5.226	0.0113*
Potential yield	3.448	0.0427*
Regrowth yield	28.003	6.77E-05*
N content in harvestable biomass	4.813	0.0172*
P content in harvestable biomass	5.338	0.0105*
K content in harvestable biomass	2.294	0.119
Mg content in harvestable biomass	1.074	0.412
Ca content in harvestable biomass	2.52	0.0964
N content in regrowth	2.212	0.156
P content in regrowth	5.794	0.0174*
K content in regrowth	5.55	0.0196*
Mg content in regrowth	28.67	6.16E-05*
Ca content in regrowth	8.016	0.00654*
Mineral N offtake in harvestable biomass	4.033	0.0298*
Mineral P offtake in harvestable biomass	1.759	0.202
Mineral K offtake in harvestable biomass	0.99	0.4495
Mineral Mg offtake in harvestable biomass	2.763	0.0771
Mineral Mg offtake in harvestable biomass	3.274	0.0494*

*Represent a significant effect of the previous crop on this variable at ($\alpha = 0.05$).

the soil at final soil sampling date did not differ from the expected value, which shows that after several years of maize cultivation with only mineral fertilization the mineralization of N from soil was very limited. In maize grown after OPM16, N_{min} in the soil was comparatively low when sampled in June suggesting some degree of immobilization had occurred during the season. However, at the end of the season (after maize harvest) the N content in soil was with 79.88 kg N_{min} ha^{-1} much higher than before the maize sowing and even exceeded the estimated mineral N by 14 kg N_{min} ha^{-1}. This indicates that after some initial immobilization in the early season, additional N was mineralized from the soil pool but could not be taken up by the maize crop due to water limitations. The same observation was made for maize following the *M. sinensis* OPM77 where mineral nitrogen in

TABLE 3 Mineral nitrogen content of soil (0–90 cm) under various treatments and calculated offtake through biomass harvest.

Previous crop	**A. Fertiliza-tion (kg N ha^{-1})**	**B. Mineral N offtake by harvested biomass (kg N ha^{-1})**	**C. Measured mineral N in soil before maize sowing (15.04.20) (kg N_{min} ha^{-1})**	**D. Measured mineral N in soil after maize harvest (22.09.20) (kg N_{min} ha^{-1})**	**E. ΔN (D − C) change of mineral N in soil from sowing until harvest (kg N_{min} ha^{-1})**	**F. Estimated mineral N in soil after maize harvest (C + A − B) (kg N_{min} ha^{-1})**	**Difference between estimated and measured mineral N in soil (D − F) (kg N_{min} ha^{-1})**
Maize	198	167.18^{ab}	18.15	44.26	+26.11	48.97	−4.71
OPM16	198	151.1^{ab}	18.15	79.88	+61.73	65.05	+14.83
OPM17	198	163.01^{ab}	18.15	49.76	+31.62	53.14	−3.73
OPM77	198	198.75^{a}	18.15	44.94	+26.79	17.39	+27.55
Switch-grass	198	146.02^{b}	18.15	17.81	−0.33	70.13	−52.31

Note: As a mixed probe was used for the first sampling date, all treatments begin at 18.15 kg ha^{-1}. Letters represent significant differences in nitrogen offtake in maize following removal of previous crops. Crops that have the same letter are not significantly different at ($\alpha = 0.05$).

the soil was 26 kg N_{min} ha^{-1} higher at the final sampling date than at the sampling before maize sowing. Interestingly, no major immobilization could be observed here at the midterm soil sampling compared to maize after maize (Figure 6) and the observed increase occurred although the N offtake by the biomass equaled the fertilizer application. This indicates an additional nitrogen mineralization from the soil or dead biomass pool of about 27 kg N_{min} ha^{-1}.

In the treatment maize following OPM17, amount of N_{min} in the soil after maize harvest was similar to the estimated value based on offtake. This indicates that for this hybrid the mineralization from the soil pool may have been compensated by the immobilization of the decaying root and rhizome biomass. Another explanation could be linked to the hybrid morphology, which was producing large amounts of thin, creeping rhizomes covering the whole plots area, while OPM16 and OPM77 produced compact shoot bunches. This led to a strong regrowth (highest shoot number amongst miscanthus hybrids) in the early season taking up mineral nitrogen, which was then died off due to effective control by the herbicide application (Figure 3).

In switchgrass, amount of N_{min} was much lower than expected, suggesting immobilization had occurred as a result of decaying residual biomass following removal. Although less N was removed from the field in the maize following switchgrass treatment than in other treatments, N_{min} was lowest in this treatment at the final sampling date and values were 52 kg N ha^{-1} lower than the estimate based on offtake data (Table 2). This would agree with the results of Ferrarini et al. (2022) who observed a greater relative immobilization from degrading switchgrass residues compared to miscanthus. Franco et al. (2018) observed that yields of wheat grown following the removal of switchgrass were lower than those of wheat grown in monoculture, and attributed this to immobilization caused by decaying underground biomass. The result seen here would corroborate that finding, however as previously stated, the decreased yield of maize following switchgrass was likely also caused by competition for water.

The results observed in the OPM16 and OPM77 treatments would appear to confirm the findings of Dufossé et al. (2014), and Mangold et al. (2019) wherein an increase in N_{min} in soil was observed following miscanthus removal. Dufossé planted wheat following miscanthus removal and yields did not differ between a control and fields where miscanthus was removed. Wheat grown after miscanthus did however have fewer stems, emerged more slowly, and had a higher harvest index. The authors suggest that residual miscanthus matter led to a wider C:N ratio (i.e., immobilization) and thus hindered growth in the beginning of the season. However, in their study a vast amount of litter biomass (32 t DM ha^{-1}) was incorporated into the soil, which was not the case in our study due to harvest in October in the years before the crop removal. No negative effect on maize yield was observed in this experiment after miscanthus removal, which may be caused by less litter incorporation into the soil and more time passed between miscanthus ploughing and sowing of a follow-on crop in this experiment than in that of Dufossé et al. (2014). Mangold et al. (2019) also planted follow-on crops shortly after miscanthus removal, and observed low yields in barley and rye grass. This may be the result of immobilization as seen in Dufossé et al. (2014); however, it is unclear as the author's noted that many barley ears had fallen from the plants prior to harvest, which would also explain the low yield they observed.

A significant difference in yield was seen after switchgrass removal, and although no significant yield difference was observed in this experiment for maize after miscanthus removal compared to the maize control, the field measurements suggest that plants were slightly smaller after miscanthus removal compared to maize in monoculture. This was more likely a result of water competition in the early growing season, which could not be compensated for later on due to drought conditions, rather than observed midseason nitrogen immobilization. However, the N immobilization in the soil might have been lower in this study, due to green harvest of miscanthus and switchgrass in the years before the removal leading to very little litter and strongly reducing the incorporation of biomass with a wide C:N ratio into the soil at removal. Effects of harvest time, litter incorporation and time delay between crop removal and sowing of follow on crop on nitrogen immobilization need to be further investigated to better understand nitrogen dynamics after removal of perennial miscanthus.

4.4 | Further considerations

Genetic differences between miscanthus and switchgrass genotypes may affect removal efficiency and the effect of removal on the soil afterwards. Withers (2015) showed that a large variety in underground morphology exists within the miscanthus genus and Chae et al. (2014) looked at morphological differences between various species of miscanthus. *M. sinensis* genotypes typically display tuft like morphology and produce small rhizomes that do not spread. *M. sacchariflorus* genotypes produce longer, spreading rhizomes that are generally larger. The novel genotypes used in this study tended to show the morphology of one of the parents as opposed to an intermediate phenotype. OPM77 thus displayed *M. sinensis* morphology, as did OPM16. OPM17 however looked like its *M.*

sacchariflorus parent. Ferrarini et al. (2022) examined the in vivo degradation of perennial biomass crops fine root matter and underground organs such as rhizomes. It was shown that fine root matter and underground plant organs have different degradation kinetics, and that different factors impact the mobilization of nitrogen from each biomass type. Mineralization of N in fine roots is strongly correlated with C:N ratio, cellulose content, and ash content whereby for underground organs such as rhizomes, total nitrogen content, the ratio of easily degradable biomass to resistant biomass and cellulose are important factors.

These observations somewhat explain the results of this experiment. OPM77 displays typical *M. sinensis* morphology and for this reason could have been easier to decay than OPM16 or OPM17. The smaller amount of belowground biomass produced by an *M. sinensis* genotype would be easier to remove via ploughing and would cause a smaller amount of immobilization. More generally the results of this experiment suggest that rhizome morphology could be a good indicator to predict nitrogen dynamics after crop removal and needs to be considered in future research.

5 | CONCLUSION

This experiment sought to determine what effect the removal of various miscanthus genotypes and switchgrass had on maize as a follow-on crop. Maize was shown to be a suitable follow on crop to miscanthus, capable of coping with volunteer regrowth without a significant yield depression occurring. This confirms the result achieved by Mangold et al. (2019).

It was demonstrated that a combination of tillage and herbicide treatment against monocots using a sulfonylurea herbicide was sufficient to suppress miscanthus regrowth in the follow-on crop maize and neither an additional glyphosate application, nor additional soil cultivation is needed to eradicate a miscanthus plantation. Generally, less rhizome forming *M. sinensis* hybrids seem to be easier to remove than hybrids producing large rhizome amounts.

Switchgrass removal was achieved as no regrowth was seen in the wheat crop grown following maize, however the maize yield in this experiment was nevertheless significantly lower than maize grown in monoculture. A different choice or an additional application of herbicides would likely remedy this problem.

Nitrogen immobilization arising from degradation of residual miscanthus biomass in the soil was not a problem in this experiment, and in some genotypes even a net mineralization was observed over the vegetation period, which could be a result of the lower quantity of litter incorporated into the soil due to green harvest in the years before the crop removal. The results here suggested that switchgrass on the other hand resulted in immobilization. Further research is needed to understand the nitrogen dynamics following removal of a spring harvested crop, especially in cases where a large amount of litter is present, to allow the farmer to adapt the fertilizer application to compensate for the immobilization caused by decaying biomass. Whether this is feasible is dependent on the fertilization ordinances in Europe. Research looking at the effect of removal on nitrogen and carbon emissions would also be valuable, as the sequestration potential of these crops is one of the strongest arguments for their cultivation (McCalmont et al., 2017).

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used in this experiment is available at https://doi.org/10.5061/dryad.vx0k6djz7.

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