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Changes in Genotype Composition and Morphology at an Experimental Site of Common Reed (*Phragmites australis*) Over a Quarter of a Century

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Abstract
The cultivation of common reed (*Phragmites australis*) is one of the most promising practices of paludiculture on fen peatlands. This highly productive grass has a high adaptation capacity via high levels of genetic diversity and phenotypic plasticity. In this study, a reed experimental site established on a degraded fen in 1996/97 with a mixture of monoclonally (meristematically propagated plantlets) and polyclonally (pre-grown seedlings) planted plots was investigated by microsatellite genotyping. All nine genotypes of the monoclonal planted plots were recovered and could be genetically characterized; invasion by other genotypes was negligible. Similarly, the polyclonal plots sustained high clonal diversity with no prevalence of a single genotype. The growth characteristics of the five quantitatively investigated genotypes significantly differed from each other ($\alpha = 0.05$): dry biomass per stem 5–18 g, panicles per m^2 20–60, average stem diameter 3.5–6 mm, height 170–250 cm. Similarly, the persistence of genotypes at the planted plots and their invasiveness (ability to invade neighboured plots) varied. These results show that common reed stands are extremely persistent even if established with genotypes that are likely not to be locally adapted. Their genetic structure remained stable for at least 24 years regardless of the planting density (1, 4, and 10 plants per m^2). Our results indicate that farmers may be able to maintain favourable genotypes for many years, thus the selection and breeding of common reed as a versatile crop for rewetted peatlands is a promising objective for paludiculture research.

Keywords Paludiculture · Peatland · Genotyping · Clonal growth · Local adaptation

Introduction

Peatlands occupy only 3% of the world's land surface (Xu et al. 2018), but accumulate more than 30% of the world's soil carbon (Scharlemann et al. 2014). As a result of continuing drainage, degrading peatlands make a significant contribution to global warming due to carbon dioxide and nitrous oxide emissions and are thus a considerable anthropogenic source of greenhouse gases (Joosten et al. 2016; Leifeld et al. 2019). Therefore, their conservation and restoration are a key element of the global carbon strategy in the land use and agriculture sectors (Taillardat et al. 2020; Tanneberger et al. 2021; Temmink et al. 2022). In terms of both environmental and economic demands on peatland management, paludiculture is a reasonable concept that allows for long-term, sustainable cultivation of wet and re-wetted peatlands (Wichtmann et al. 2016; Martens et al. 2022). Apart from being an effective carbon store, wet peatlands can also be a valuable source of biomass as raw material, fuel, food and medicine; in addition, they can be used for water retention and purification from nutrients and pollutants (Biancalani and Avagyan 2014; Wichtmann et al. 2016; Bonanno et al. 2018).

One of the most suitable and frequently used wetland plants for paludiculture and phytoremediation is common reed, *Phragmites australis* (Cav.) Steud. (Rezania et al.

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2019; Geurts et al. 2020; Lahtinen et al. 2022). *Phragmites australis* is a large perennial grass (Poaceae), with high levels of peat formation. Its biomass is rich in cellulose and hemicelluloses, which can be used in the production of alcohols, biofuel (Sathitsuksanoh et al. 2009; Dragoni et al. 2017; Eller et al. 2020; Czubaszek et al. 2021), pulp and paper (Brix et al. 2014). Winter harvested common reed serves traditionally as construction, insulation and roof thatching material (Köbbing et al. 2013; Becker et al. 2020). Further innovative utilisation options may be discovered or expanded in the future, like the unique 3D silicon structure in common reed leaves suitable for electrochemical performance in Lithium-Ion batteries (Liu et al. 2015).

Common reed is a cosmopolitan species that occupies a wide ecological niche and often forms monodominant stands. It has an extensive root system capable of spreading vegetatively over an area of more than 100 m^2 and penetrating to a depth of up to 2 m, which allows the plant to regrow shoots every spring and after mowing (Douhovnikoff and Hazelton 2014; Packer et al. 2017). Common reed can also propagate sexually, with seed viability ranging from 70 to 100% (laboratory experiments (Packer et al. 2017). However, sexual reproduction is considered rare, especially in already established sites (Engloner 2009; Packer et al. 2017), but is crucial for establishing new populations in nature (Albert et al. 2015). According to Koppitz et al. (1997), there are three stages of common reed stand development: (1) "settlement" (seed germination prevails), (2) "propagation and establishment" of clones (the best adapted genotypes start to propagate vegetatively), and (3) "stationary" (only vegetative propagation of a few, most competitive, genotypes). Thus, genetic diversity tends to decrease in common reed populations over time due to the transition from sexual to vegetative propagation of one or a few clones. In general, the level of genetic diversity influences the fitness and adaptive potential of a population, especially in stressful environments (Keller 2002; Markert et al. 2010). Therefore, monoclonal populations of *P. australis* are assumed to be more at risk of environmental stressors than common reed stands with a higher number of clones because of their lower genetic diversity (Koppitz et al. 1997).

For the successful establishment and maintenance of a common reed stand for paludiculture and to prevent its dieback, information on its levels of genetic diversity is crucial. It is also important to estimate the time during which selected genotypes with favourable features can persist after planting. So far, only scarce information about the population genetic dynamics of common reed is available, mostly about natural stands (Koppitz et al. 1997; Lambertini et al. 2008), and no controlled, long-term research has yet been conducted. In this study, we investigated changes in genetic diversity and growth at an experimental site of *P. australis* 24 years after its establishment. Our main objective was to assess how genetic diversity changed during this time period and whether the use of different numbers of genotypes and planting densities for the establishment of the common reed stand led to different levels of genetic diversity. We also aimed to reveal variations in morphology and occupied areas between genotypes in order to find possible differences in morphological traits and propagation strategies.

Materials and Methods

Sampling Site

The experimental site was established in 1996 and 1997 on degraded fen grassland near Biesenbrow, Germany (N 53.11234°, E 14.02670°). An area of 8 ha was separated from the drained surroundings, ploughed and harrowed to destroy the grassland vegetation to provide favourable conditions for common reed establishment (Timmermann 1999). Gradual rewetting was conducted by ensuring high ditch water levels via weirs and pumping ditch water into elevated water reservoirs. Due to the slightly sloping surface, the intended water management encompassed two approaches: (a) trickling in the elevated parts with water levels below ground and (b) inundation in the lower parts with water levels from 5 to 50 cm above ground (in a west-east direction) (Timmermann 1999). Fluctuating water levels and dryer periods were observed over the total life time of the experimental site. Water management was carried out in coordination with ongoing research projects (1997 to 2002; 2011 to 2013; Maassen et al. 2015) and stopped in 2015 (Christine Schmidt, manager of the water board "Welse", pers. comm. 08.05.2018). No regular biomass harvest was conducted with the exception of one maintenance cut, which took place in preparation for a flooding experiment with treated wastewater in 2011 (Maassen et al. 2017).

To set up the common reed experimental site, the area was divided into differently sized (from 12.5 × 12.5 m to 25 × 25 m) and shaped plots (squares, rectangles irregular hexagons) that were intended as polyclonal plantings with several unique genotypes per plot and monoclonal plantings with only one of nine genotypes per plot (Fig. 1). For the polyclonal plantings, each plot was established with one of three different techniques (Timmermann 1999): (a) by planting pre-grown plants in the greenhouse, (b) by direct sowing of panicles, and (c) via planting of stem cuttings. The first technique was the most successful with a mortality rate of less than 1%, while the other two methods had very high mortality rates (> 95%). All plots included in our study (n = 66) were established by planting pre-grown plants. Two planting strategies were used (Timmermann 1999):

monoclonal (only one of nine genotypes per plot) and polyclonal (several unique genotypes per plot). The seeds were collected in three different locations giving three different ecotypes ("A" = "Greiffenberg", "B" = "Landin", "C" = "Peene bei Anklam") at the density of 1, 2, or 4 plants per m^2 (Timmermann 1999). The term "ecotype" is used here following the previous studies of the experimental site referring only to the origin of genotypes, and no local adaptation essay was conducted. For the monoclonal plantings, nine regional genotypes ("1" = "Ries", "3" = "Landin", "4" = "GreiffA", "5" = "GreiffB", "6" = "GreiffC", "7" = "GreiffD", "8" = "Sedd1", "9" = "Mueggk", "10" = "PAR1") were meristematically propagated and planted with densities of 1, 4, or 10 plants per m^2 (Koppitz et al. 1999, 2000). Genotypes "3" through "7" were collected from the vicinity (~8 km) of the experimental site, and genotypes "1", "8", "9", and "10" were collected from more remote locations in northeast Germany (~22, 80, 80, and 100 km from the experimental site, respectively). Some areas were left open after ploughing for free succession. For our study, 50 monoclonal plots, 6 polyclonal plots, and 10 plots for free succession were chosen.

Genetic Analysis

Samples for genotyping were collected in September 2020 (from the area established in 1996) and June 2021 (from the area established in 1997). Fresh leaves from one shoot were taken every 2 m along transects (Fig. 1) and preserved in silica gel. Transects were located in the centre of the plots, covering the plots with each of nine monoclonally planted genotypes and planting densities of 1, 4 and 10 plants per m^2, giving 41 studied plots. Similarly, all three ecotypes from the polyclonal plots were sampled for planting densities of 1 and 4 plants per m^2 (6 plots, in total). Some transects were located within ten free succession plots. One plot (monoclonally planted genotype ''8'', 1 plant per m^2) was sampled at greater depth, collecting leaves every 0.5 m along three transects. In total, 387 samples were collected and genotyped for the genetic analysis. Additionally, two specimens of genotypes "1" and "7" were collected from the research station "Paulinenaue" (Brandenburg, Germany). These two specimens were the only available references for monoclonally planted genotypes.

For all samples, DNA extraction, polymerase chain reaction (PCR) for eight single sequence repeat (SSR) markers (using two multiplexed sets of primers) and clone assignment were conducted. Only samples which matched each other in all alleles of analyzed SSR loci were assigned to the same multilocus genotypes (hereafter simply referred to as genotypes). For all genotypes, principal coordinate analysis (PCoA) was performed based on Bruvo distance (Bruvo et al. 2004) calculated with the function *meandistance.matrix2* in the R package *polysat* 1.7-7 (Clark and Jasieniuk 2011). For monoclonal plots, the most frequently encountered genotype was assumed to be the one originally planted. For these genotypes, the *trnT*(UGU) – *trnL*(UAA) chloroplast region was sequenced and haplotypes were assigned as in Saltonstall (2016). All above procedures were conducted according to Kuprina et al. (2022).

To estimate genetic diversity and clonal variation, the gene diversity index (the probability that two randomly selected samples have different genotypes) (Nei 1987) was calculated using Arlequin 3.5.2.2 (Excoffier and Lischer 2010). The change in gene diversity was estimated by comparison of the original (according to the establishment scheme of the experimental site) and current values of gene diversity. The values of gene diversity for plots, established with different strategies and densities, were also compared.

For each of the nine monoclonally planted genotypes, two parameters of relative competitiveness were calculated: (1) persistence, the proportion of samples with each genotype on all samples in the respective monoclonal plots; and (2) invasiveness, the proportion of samples with each genotype on all samples taken outside the respective monoclonal plots. For these calculations, only samples from originally monoclonal plots were included, using five samples per plot.

Morphological and Biomass Analysis

To compare the growth and biomass production of the nine monoclonally planted genotypes, additional sampling was conducted in October 2022. For each of nine genotypes, the above-ground biomass was harvested from two plots in a pair of 0.5 m^2 squares on each plot, giving 18 plots with 36 squares, in total (Fig. 1). Because of the possible difference in water and nutrient availability along the experimental site, studied plots located as far apart as possible (in a southwest-northeast direction; Fig. 1). Only plots with a planting density of 1 plant per m^2 were studied. In each square, the number of stems and panicles, and the percentage of stems with panicles were recorded, and stem height (from the panicle tip to the cut above the ground) and width (diameter of the middle of the first intact basal internode, in two replicates) were measured. To estimate the dry biomass weight, the harvested plants were dried at 70 °C for 3 days. The dry biomass of one stem was calculated by dividing the dry above-ground biomass by the number of stems. To assign the squares to genotypes, one leaf each from five randomly chosen stems per square were preserved in silica gel and genotyped (n = 180) with primer set two according to Kuprina et al. (2022); the results of this genotyping were used only to select squares appropriate for the morphological and biomass comparisons and were not included in the

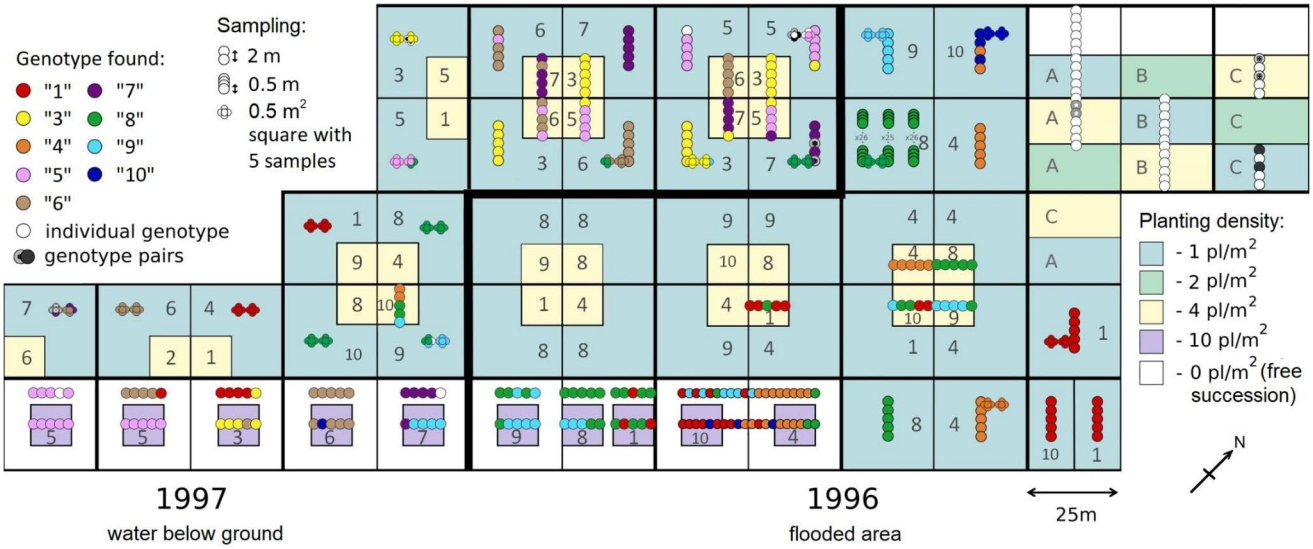

Fig. 1 Schematic of the *Phragmites australis* experimental site, established in 1996 and 1997. A closed area of the same colour indicates a plot on which either one of the nine genotypes (numbers 1 and 3–10: monoclonal plots created with meristematically propagated plants), one of the three ecotypes (letters A – C: polyclonal plots created with pregrown seedlings), or nothing (white plots) was planted. Circles represent the distribution of investigated samples (2020–2022, n = 567) and the circle colours represent the genotypes found. The thick line shows the boundary between plots established in 1997 and 1996 and between two water regimes. More information about site establishment can be found in Timmermann (1999) and Koppitz et al. (1999)

genetic analysis. Only squares with all five stem samples belonging to the same genotype were included in this analysis as representatives of one of the genotypes. The squares with more than one detected genotype were excluded. As a result, 19 squares out of 36 and five genotypes ("1", "3", "6", "8" and "9") out of nine were selected for morphological and biomass comparisons (Table 1). For each of these five genotypes, the set of selected squares contained squares from the areas established in both 1996 and 1997.

Data Analysis

Statistical tests and data visualization were performed in R 4.2.0 (R Core Team 2022) using RStudio IDE 2021.09.2 (RStudio Team 2020) and packages *ggplot2* 3.3.6 (Wickham 2016) and *vegan* 2.6-4 (Oksanen et al. 2019). Values of gene diversity were calculated using Arlequin 3.5.2.2 (Excoffier and Lischer 2010). Before analyzing morphological parameters, data were tested via the Shapiro-Wilk normality test and Bartlett test of homogeneity of variances. Normally distributed data with homogeneous variances (dry aboveground biomass, dry biomass of one stem, number of panicles and percentage of stems with panicles) were analyzed via an analysis of variance (ANOVA) with the Tukey's honestly significant difference (HSD) test (Tukey 1949). Data not meeting test assumptions (stem width, length and density) were analyzed via a Kruskal-Wallis test with Dunn's post-hoc test and Bonferroni p-value correction using the RStudio package *rstatix 0.7.0*. (Kassambara 2021).

Results

Genetic Analysis

Genotyping of samples collected along transects (2020–2021) revealed a total of 50 genotypes. As expected, nine genotypes showed the highest frequencies and accounted for 92% of all samples (Fig. 1). They were not found in the plots established by polyclonal planting and their distribution over the plots allowed the assignment to the nine previously monoclonally planted genotypes (Fig. 2): among the samples from plots with the same genotype planted, one genotype makes up for at least 68% of the samples (except for plots with genotype "10" planted). Additionally, the genotyping of two reference specimens representing genotypes "1" and "7" collected from the research station "Paulinenaue" fully matched and supported our genotype assignment. Results of a PCoA did not reveal a notable clustering of genotypes (Fig. 3). Least similar is genotype "5".

The values of original and current gene diversity were nearly identical across plots and planting strategies (mono- and polyclonal) (Fig. 4a). The numbers of genotypes identified in the plots established by mono- and polyclonal planting varied: 11 (n = 255) vs. 31 (n = 34), respectively. Gene diversity was lower for monoclonally planted plots than for polyclonally planted (0.8844 ± 0.0045 vs. 0.9947 ± 0.0086, respectively) (Fig. 4a). However, the density of the establishment had no influence on the level of gene diversity.

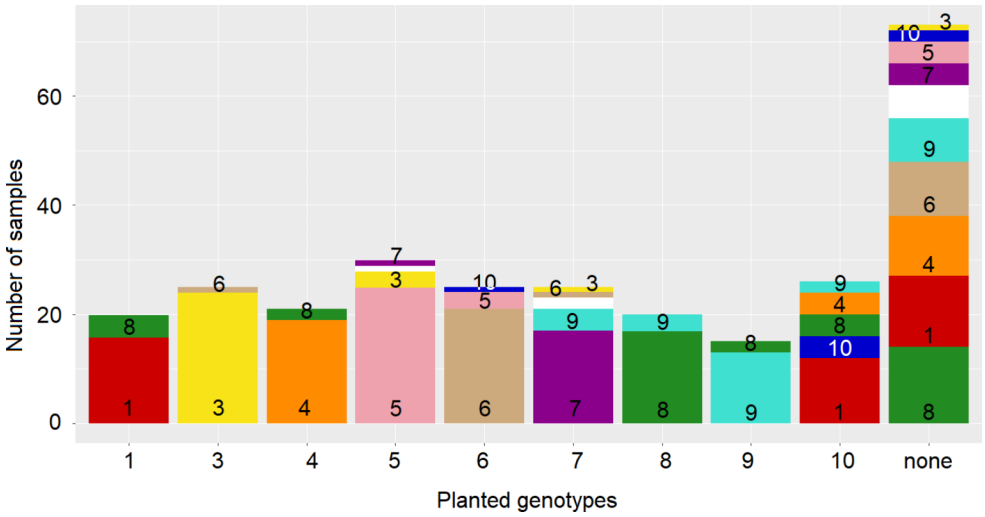

Fig. 2 Distribution of *Phragmites australis* genotypes revealed after 24 years in experimental site plots with monoclonally planted genotypes ("1", "3"-"10") and adjacent plots with free succession ("none"). Colour codes for the genotypes are the same as in Fig. 1; white depicts not planted genotypes. Numbers and colours refer to the genotypes

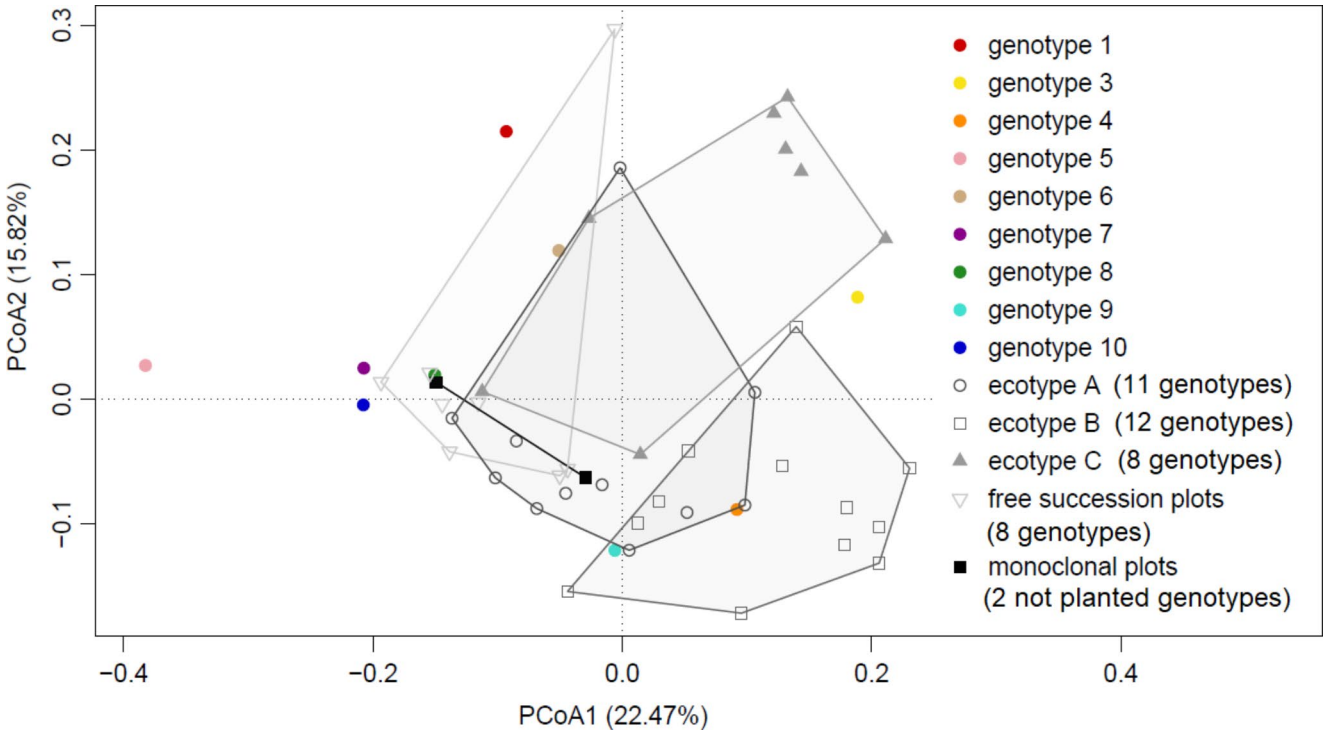

Fig. 3 Ordination diagram of principal coordinate analysis (PCoA) with the first two axes calculated from Bruvo distances for 50 genotypes of *Phragmites australis* found in 2020–2021 in mono- and polyclonally planted plots, and free succession plots (nothing planted), in the experimental site established in 1996/1997. Polygons circumscribe the distribution of genotypes in the two-dimensional plane

Genotypes showed different relative competitiveness (Fig. 4b): genotypes "3" and "4" had the highest persistence values (0.96 and 0.95, respectively), genotypes "1" and "8" were the most invasive (0.24, both), while genotype "10" had the lowest values for both parameters (0.16 and 0.02, respectively).

For the nine monoclonally planted genotypes, four different haplotypes were found: haplotype T4b for genotypes "3", "9", "10"; T5c for "5", "6", "7"; T5f for "1"; T7c for "8".

Morphological and Biomass Analysis

The comparison of five genotypes selected for the analysis (see 2.3) showed that stem width, height and dry biomass per stem varied significantly (Kruskal-Wallis test for both stem width and height: $df = 4$, $p \leq 0.0001$; ANOVA for dry biomass per stem: $df = 4$, $F = 13.1$, $p = 0.0001$) (Figs. 5a and b and 6a; Table 1). On average, genotypes "3" and "9" had the lowest values of stem width, height and dry biomass per stem, and genotypes "6" and "8" had the highest values.

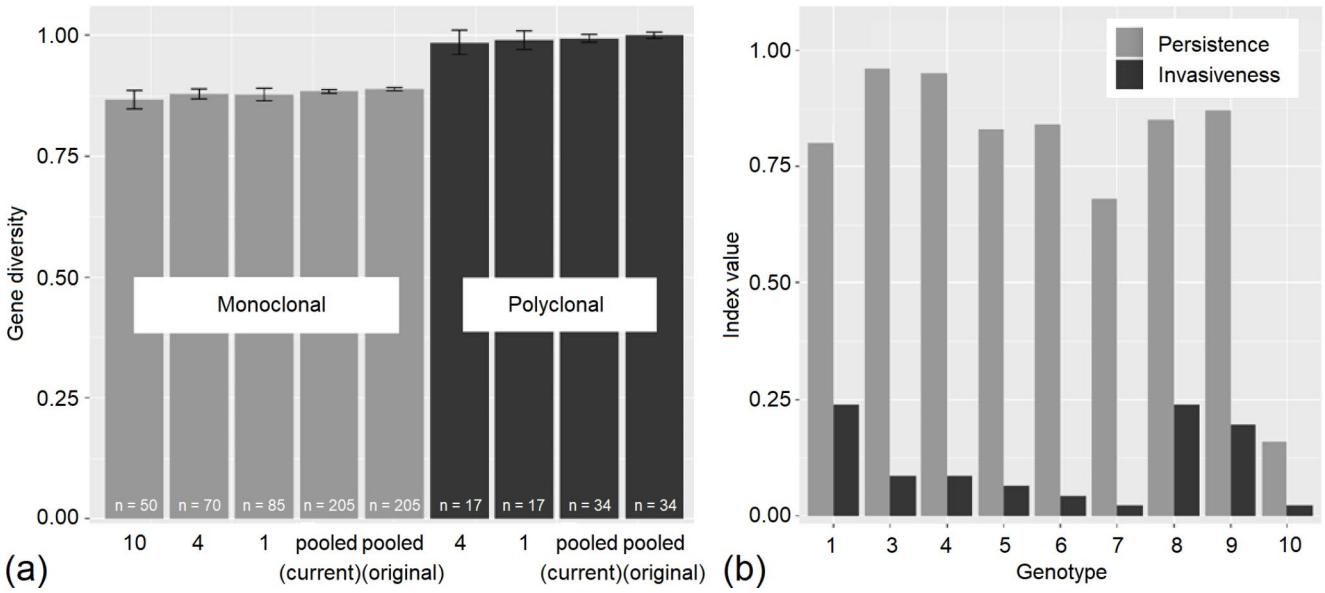

Fig. 4 (**a**) Gene diversity with standard deviations for plots with *Phragmites australis* established 24 years ago by mono- and polyclonal planting with different densities. Numbers 1, 4, and 10 denote plots with the respective planting density per m^2; pooled (current) the values found, pooled (original) - the theoretical value directly after planting as the mean of all plots. (**b**) Values of relative persistence and invasiveness for nine genotypes of *P. australis* 24 years after the establishment by monoclonal planting

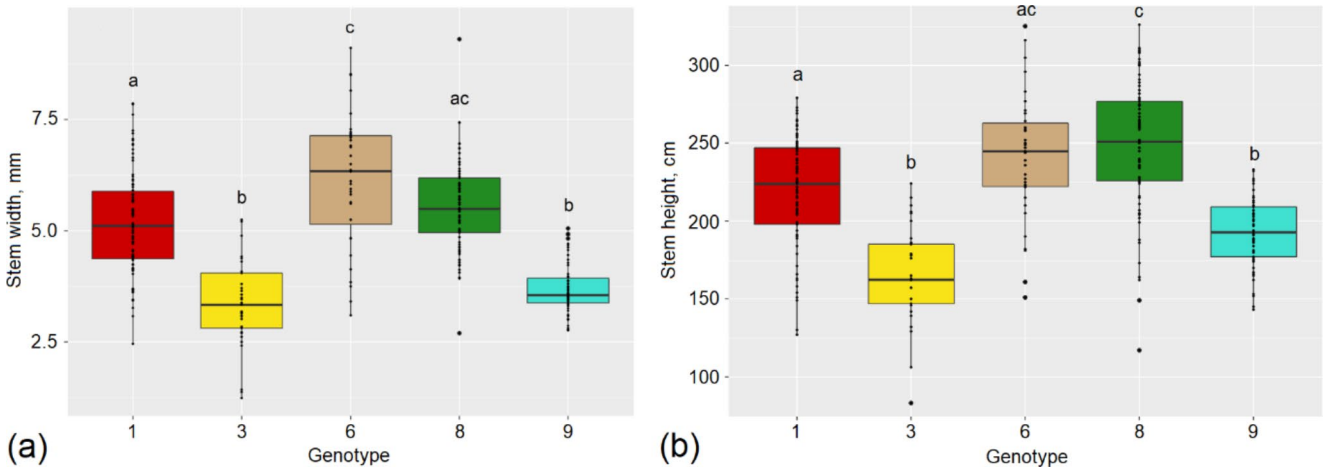

Fig. 5 (**a**) Stem width and (**b**) **h**eight of five *Phragmites australis* genotypes planted in 1996/1997 and measured in October 2022. Genotypes with the same letter code are not significantly different (Dunn's post-hoc test for the Kruskal-Wallis test with a Bonferroni *p*-value correction, $\alpha = 0.05$)

Number of panicles and the percentage of stems with panicles varied among genotypes (ANOVA, $p = 0.039$ and 0.023, respectively) (Table 1): Tukey HSD method revealed a significant difference between two genotypes: genotype "9" had higher values than genotype "6" (Fig. 6b). Differences in dry above-ground biomass (mean = 836.2 g/m^2) and the number of stems (mean = 80.3 stems per m^2) between genotypes compared by Kruskal-Wallis test were not significant (Table 1).

Discussion

Genetic Analysis

The genetic composition of the common reed experimental site did not change considerably after 24 years, and the difference in clonal variation between mono- and polyclonal plots remained. The number of genotypes in the plots established by monoclonal planting increased from 9 to 11, with the two new genotypes found only in three out of 327 samples in two plots with planting density of 1 plant per m^2 (Fig. 1). These two genotypes most likely invaded the

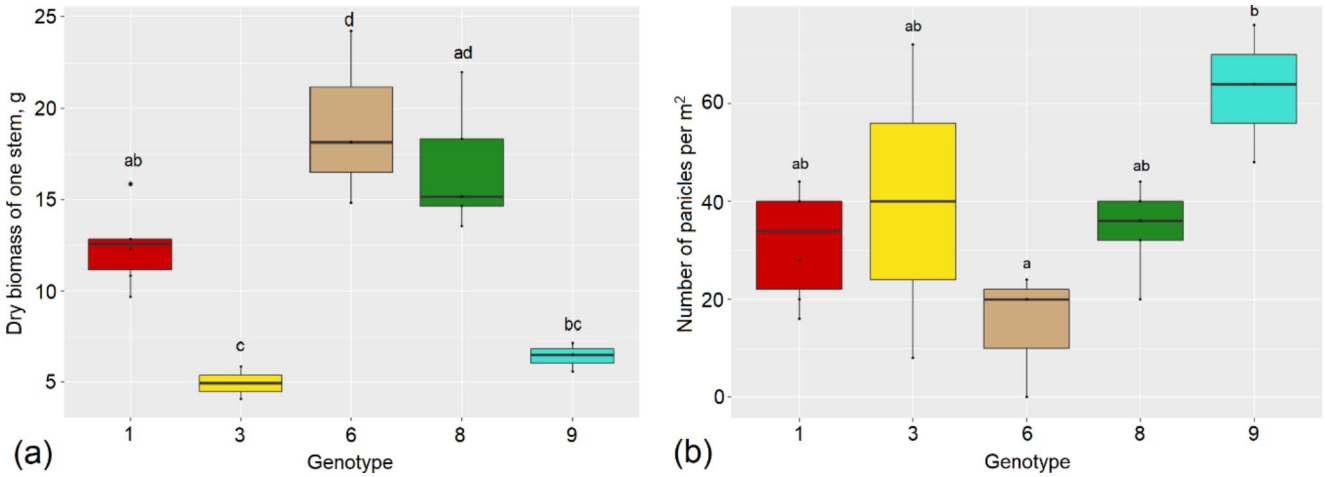

Fig. 6 (**a**) Dry biomass per stem and (**b**) number of panicles for five *Phragmites australis* genotypes planted in 1996/1997 and measured in October 2022. Genotypes with the same letter code are not significantly different (Tukey HSD test, $\alpha = 0.05$)

Table 1 Mean ± standard deviation of morphological and biomass parameters for five *Phragmites australis* genotypes monoclonally planted 24 years ago and measured in October 2022

	Genotype				
	"1" – "Ries"	"3" – "Landin"	"6" – "GreiffC"	"8" – "Sedd1"	"9 - "Mueggk"
Stem width (mm)	5.19 ± 1.17	3.35 ± 0.99	6.17 ± 1.49	5.55 ± 1.00	3.69 ± 0.58
Stem height (cm)	218.1 ± 36.1	165.3 ± 31.2	241.5 ± 40.7	248.5 ± 41.5	192.4 ± 22.4
Dry biomass of one stem (g)	12.4 ± 2.1	4.9 ± 1.2	19.1 ± 4.8	16.7 ± 3.4	6.4 ± 0.8
Dry above-ground biomass (g/m^2)	998.8 ± 520.0	621.4 ± 603.2	897.2 ± 435.6	1041.2 ± 216.0	622.4 ± 107.2
Stems per m^2	81.3 ± 40.2	114.0 ± 93.3	45.3 ± 15.1	62.4 ± 6.7	98.7 ± 23.1
Number of panicles per m^2	31.2 ± 11.6	40.0 ± 45.2	14.8 ± 12.8	34.4 ± 9.2	62.8 ± 14
Stems with panicles (%)	41.4 ± 12.0	40.0 ± 16.5	42.9 ± 23.6	54.6 ± 11.9	63.9 ± 5.8
Number of studied 0.5 m^2 squares	6	2	3	5	3

experimental site via seed recruitment. As such, established monodominant stands of *P. australis* seem to be extremely persistent. Nearly the entire site was a monodominant stand of *P. australis*, with the exception of the most northwestern plots, where common reed shared dominance with *Phalaris arundinacea*. However, despite the different composition of vegetation, no sign of a seed recruitment was found in the northwestern monoclonal plots (no not planted genotypes were found).

The plots established by polyclonal planting lost some genetic diversity due to vegetative growth, but not a substantial amount. Not all of the monoclonally planted plots received the genotype best adapted to local conditions, but the establishment advantage of the planted genotype far outweighs any possible competitive advantage of a newly invading genotype. We believe that common reed in the monoclonally planted plots have not yet reached the "stationary" stage and is still undergoing the stage of "propagation and establishment" with several genotypes extensively growing vegetatively, whereas the plots established by polyclonal planting are in the very early phase of the "propagation and establishment" stage with only a few genotypes starting to grow vegetatively.

Apart from the difference in the original number of genotypes between plots with different establishment strategies, the variation in gene diversity could be influenced by the mostly higher water level at the polyclonally planted plots in comparison to the monoclonal plots (all the samples from polyclonal plots were collected from the flooded area). However, for the monoclonal plots, no clear tendency towards an increase in genetic diversity with lower water level was detected. It's possible that insufficient difference in water level across the site combined with the high plasticity of common reed resulted in no influence of water regime on genetic structure. Another explanation of the high gene diversity in the polyclonally planted plots can be the genetic and/or epigenetic similarity of multiple genotypes propagated from seeds with the same origin, which may lead to similar ecological preferences and, therefore allows coexistence. Interestingly, the result of PCoA did not show a genetic clustering of genotypes with the same ecotype (but did not exclude a possible epigenetic similarity). It also did not indicate the clustering of monoclonally

planted genotypes according to their geographical origin, proving again the high level of gene flow and the presence of a large interbreeding metapopulation of common reed in northeast Germany (Lambertini et al. 2008; Kuprina et al. 2022). Therefore, we can conclude that the high genetic diversity detected for the polyclonally planted plots is most likely due to the originally high number of genotypes, and is unlikely attributed to varied environmental conditions or ecotype origin.

There is no available information about the "window of opportunity" allowing the establishment of different genotypes in a natural population of *P. australis*. Lambertini et al. (2008) compared the clonal variability of eight natural common reed stands in the Po Plain, Italy. They described one monoclonal population with a low disturbance level which was at least 50 years old and seven polyclonal populations with a high level of disturbance and an estimated age of about 30 years. It is not yet clear whether population age or level of disturbance has a greater impact on the genetic structure of a common reed population. In addition, effective population size, mutation rate, and linked selection (Ellegren and Galtier 2016) contribute to the genetic structure, which greatly complicates investigation of genetic changes in natural common reed stands over time. Although disturbance is suggested to be a driver of seed recruitment and genetic diversity for common reed (e.g., Lambertini et al. 2008b; Kettenring et al. 2011), most studies comparing levels of genetic diversity in natural common reed populations did not find the expected increase of genetic diversity under moderate disturbance (Fant et al. 2016; Kuprina et al. 2022). Therefore, we assume that an undisturbed population, like the experimental site studied here, will reach the "stationary" stage not earlier than 50 years (Lambertini et al. 2008), and possibly much longer. This assumption provides paludiculture farmers the expectation of being able to either maintain favourable genotype(s) or preserve high genetic diversity in cases without genotype preference for many years. However, the effect of disturbance by regular mowing on the population genetic structure of a recently established common reed stand has yet to be studied.

This experimental site was also studied in 2001, 4–5 years after establishment. Growth parameters, biomass and C/N content of stems were measured for ten plots established in 1997 monoclonally with a density of 4 plants/m^2. Clonal variability (genotyping) was also explored using RAPD-PCR with primers M13 and $(GACA)_4$ (Koppitz and Buddrus 2004). Clone composition of both mono- and polyclonal plots was studied via 10 randomly selected samples from one plot of each genotype and ecotype. For three ecotypes, three water levels were also compared (dry, wet and flooded). The observed patterns of genetic diversity were similar to those found in our current study (2020–2021). In 2001, nearly all analysed samples for polyclonally planted plots represented different genotypes, despite the difference in planted ecotypes and water level. Monoclonally planted plots, however, already showed a change in number of genotypes: plots contained from one (plots with genotypes "3", "4" and "7" planted) to four (plot with genotype "8" planted) different genotypes per five investigated samples per plot. In our study, genotypes "3" and "4" also showed the highest levels of persistence, but genotype "10" demonstrated the lowest persistence (Fig. 4b). The levels of persistence and invasiveness of monoclonally planted genotypes do not show connection with the position of these genotypes on the ordination diagram of PCoA, as well as with their assignments to haplotypes. That may indicate a non-genetic source of differences in invasiveness and persistence between genotypes.

We expected genotypes "3", "9" and "10", which have haplotype T4b (widely known as M when combined with haplotype R4b of chloroplast marker *rbcL – psaI* (Saltonstall 2016), to have the highest values of invasiveness. Studies describing invasive lineages of *P. australis* in North America, China and Australia revealed haplotype M as the most common for these lineages (Saltonstall 2002; Lelong et al. 2007; An et al. 2012; Hurry et al. 2013); haplotype M is also the most common haplotype in Europe (Lambertini et al. 2012) and northeast Germany (Kuprina et al. 2022). However, genotype "10" had the lowest values of persistence and invasiveness, which is not surprising, since it originated from a mesotrophic clear water lake (Parsteiner See, Germany) where it already displayed low productivity and short culms (Zemlin at al. 2000).

Our experimental site can be categorised as a nutrient rich area. It was intensively used as arable land for growing maize and fodder grasses with high fertilizer input since the mid-1970's, and with reduced intensity as grassland use since 1992 (Timmermann 1999). The nutrient availability due to peat degradation is high. All the other genotypes originated from eutrophic lakes or meadows that were temporarily or Permanently flooded, especially genotype "1", which originated from another extreme habitat: floodable fields (near Blankenfelde, Germany) with high pollution of heavy metals and nutrients (Koppitz et al. 2000). Therefore, the local adaptation of genotypes is essential for their successful establishment and persistence, and might persist over multiple years. For paludiculture it is thus important to investigate site properties and select suitable genotypes to ensure successful establishment of a competitive, long-lasting reed culture.

Morphological and Biomass Analysis

Under optimal water supply and the absence of competing species, common reed can display impressively rapid expansion right after planting (Kühl 1999; Timmermann 1999). Already in two years following polyclonal planting (1 plant per m^2), common reed covered more than 90% of the plot's surfaces in our experimental site (Timmermann 1999). It was shown for another common reed stand (Vymazal and Krőpfelová 2005), that a maximum of biomass production can be reached in three to five years, and that there is a tendency to gradually increase stem density for at least the next five seasons, perhaps as a result of competition between shoots. For natural populations, mature common reed stands (older than 50 years) have about six times lower stem density than young populations, perhaps due to litter accumulation and decreased light availability (Packer et al. 2017). However, in our site, despite plots with different planting densities being investigated in 2001 and 2022, the number of stems per m^2 (taken as the average per genotype) was comparable between assessment years: 2001 showed 54–114 stems per m^2 for plots with 4 plants/m^2 and 2022 showed 46–156 stems per m^2 for plots with 1 plant/m^2; (Table 1; Koppitz and Buddrus 2004).

Stem width and height, dry above-ground biomass per stem, as well the absolute and relative number of panicles varied among genotypes. Interestingly, genotypes "3" and "9" (both showing haplotype T4b) had the shortest, but the densest (not significantly) stems. The morphological comparison of these five genotypes made in September 1998 (Koppitz et al. 2000) showed that genotypes "3", "6" and "8" had the tallest stems, and the genotypes "3" and "9", like in our study, had the densest stems. However, the measurements from the summer of 2001 (Koppitz and Buddrus 2004) revealed that genotype "1" had the shortest stems, while genotype "3" had the densest with genotype "8" the least dense stems. We did not find significant differences in dry above-ground biomass and number of stems between five studied genotypes, although in 2001 genotype "6" produced more biomass, than the other genotypes (Koppitz and Buddrus 2004). These results represent a change over the 20 years between studies and show that some genotypes consistently follow a physiological strategy, producing either more shoots of smaller size (like genotypes "3" and "9") or less shoots with bigger size (genotype "8"), leading to a comparable above-ground biomass. It is also reasonable to assume that the phenotypic manifestation of the genotype is dynamic, changing over time and under different climatic conditions. We conclude that only long-term experiments under controlled conditions (mesocosms) can be used to select a genotype with favourable performance in specific environmental conditions.

Several studies describe a high level of phenotypic plasticity in *P. australis* (Hansen et al. 2007; Achenbach et al. 2012; Eller and Brix 2012). In changing environments, common reed has a primary strategy to alter its morphology, but not its phenological or reproductive traits (Ren et al. 2020). For example, in the first years following the establishment of our experimental site, the same genotypes in the dry plots produced less than half of the biomass than they did on the flooded plots (Koppitz et al. 2000). This demonstrates a higher biomass for *P. australis* when water is plentiful. Genotypes can also vary both in morphology and biomass production after being transplanted into similar environments, and this variation was explained by difference in genetics (Hansen et al. 2007; Achenbach et al. 2012; Haldan et al. 2023). Additionally, the demethylation of *P. australis* originating in freshwater, but not in saltwater populations, can improve growth under salt stress (Song et al. 2022). However, it is not yet known how large the contribution of epigenetics to the phenotypic plasticity of common reed is. It is safe to assume that both genetics and epigenetics have an impact on local pre-adaptation and phenotypic traits.

Lessons Learnt for Common Reed Paludiculture

Harvesting natural common reed stands is a traditional paludiculture. Using common reed for thatching has the highest added value compared to energetic use by direct combustion or biogas generation, but also the highest demand for biomass quality (Wichmann 2017). Selection and breeding might improve morphological or phenological characteristics for thatching reed. For instance, genotypes losing the leavers earlier in winter are advantageous by prolonging the harvesting season, allowing a higher machine utilisation and thus reducing harvesting costs. Our results indicate that (a) planted reed stands can persist over a quarter of a century, (b) genotypes need to be suitable for the conditions of the selected site (e.g., water and nutrients), (c) favourable genotypes may be maintained for many years and e) desired morphological characteristics may persist. Therefore, the selection or breeding of common reed can be justified to further explore and develop paludiculture for thatching but also for other, including not yet known, high-value applications. A major question for further research is the long-term effect of annual mowing on establishment, development and persistence of favourable genotypes.

Conclusion

We have demonstrated that the experimental site conserved both the original level and patterns of clonal variation of *P. australis* over 24 years. Plots established by the monoclonal

planting of nine genotypes predominantly contained the original genotypes (99% of the samples). Polyclonally planted plots remained mostly polyclonal (82% of the samples were found as singletons). Therefore, even stands established with different genotypes, likely not all locally well adapted, remain nevertheless extremely stable. Planting density did not have an influence on subsequent gene diversity (total number of genotypes) for either establishment strategy (mono- or polyclonal). All genotypes planted monoclonally remained despite differences in persistence and invasiveness. A comparison of the performance of five morphologically assessed genotypes revealed differences in multiple traits, including stem width, height, and dry aboveground biomass per stem, as well as in the absolute and relative number of panicles. Many morphological differences between genotypes observed here did not correspond to the measurements obtained during previous studies conducted in the second and fifth year following the creation of the experimental site, indicating high plasticity of *P. australis* genotypes over time and emphasizing the necessity of long-term, controlled experiments for the selection of favourable genotypes. Moreover, some genotypes showed a tendency to follow different morphological trends for several years, which justifies the importance of genotype selection for common reed. Here we can conclude that established genotypes of *P. australis* can persist for many years and potentially retain the desired morphological characteristics for paludiculture.

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Data Availability The datasets generated and/or analysed during the current study are available from the corresponding author upon request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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