




Article

Mortality of Different *Populus* Genotypes in Recently Established Mixed Short Rotation Coppice with *Robinia pseudoacacia* L.

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Abstract: Short rotation coppices play an increasing role in providing wooden biomass for energy. Mixing fast-growing tree species in short rotation coppices may result in complementary effects and increased yield. The aim of this study was to analyze the effect on mortality of eight different poplar genotypes (*Populus* sp.) in mixed short rotation coppices with three different provenances of the N-fixing tree species black locust (*Robinia pseudoacacia* L.). Pure and mixed stands were established at two sites of contrasting fertility. Survival of poplar was assessed for each tree two times a year, for a period of three years. In the first two years, high variation in mortality was observed between the genotypes, but no significant differences between pure and mixed stands were identified. However, three years after planting, higher mortality rates were observed in the mixtures across all poplar genotypes in comparison to pure stands. The expected advantage on growth of combining an N-fixing tree with an N-demanding tree species, such as poplar, was overshadowed by the *Robinia*'s dominance and competitiveness.

Keywords: short rotation coppice; poplar; black locust; survival rate; mortality; mixing effect

1. Introduction

Wood is a renewable source of energy with great potential as a fossil fuel substitute [1]. Besides forest stands, short rotation coppices on arable lands are increasingly important as sources of wood biomass for energy [2]. To avoid rivalry between areas for food and wood production, short rotation plantations are often established on marginal lands where soil nitrogen and water are limiting [3]. However, the yield and fitness of *Populus* sp., the genus most commonly used for short rotation wood production in Germany, are highly correlated with nitrogen and water availability [4,5]. Fertilization is commonly employed, but it is strongly linked to nitrate pollution of groundwater [6–8]. Therefore, it is recommended that fertilizer use for poplar plantations be decreased. An alternative to the use of fertilizer may be the simultaneous planting of legumes. Due to their ability to fix nitrogen, these plants can potentially increase soil fertility without chemical fertilization [9–11]. It was shown that tree species combinations with complementary ecological traits might have the potential of higher yield compared to monocultures [12,13]. For instance, mixing N-fixing tree species may facilitate the growth of neighboring trees that demand nitrogen [14,15]. Oliveira et al. [16] and Rédei et al. [17] were in fact

able to prove that mixing black locust as a complementary tree species to white poplar might, under certain circumstances, increase poplar's biomass production.

Besides having the ability to enrich the soil with nitrogen, black locust has other promising qualities, such as rapid growth-rate, high tolerance for low fertility sites, and high resistance to drought [11,18–20]. However, as a robust pioneer tree, black locust has a high potential for propagation and dispersal in open landscape and may turn invasive on sites of limited nutrient and water availability [20]. Still, the black locust is widely used in Germany in short rotation coppices on such sites [20]. Naturally, unlike poplar, *Robinia* short rotation coppices do not require nitrogen fertilizers and are managed as low-input systems [11].

Combining a fast growing tree species such as poplar with N-fixing species such as black locust may contribute to increased aboveground biomass production [16,17]. Theory suggests that the more diverse the tree species in a mixture, in terms of resource demand and foraging strategy, the higher the complementarity effects [13]. Encouraging examples of successfully cultivated mixed cropping of N demanding, fast growing species with N-fixing tree species have been reported, e.g., by Hansen and Dawson [15] and Forrester et al. [21].

The survival of poplar in the early stages of mixed cropping is influenced by a number of abiotic (drought and frost) and biotic factors (weeds, fungi, bacteria, viruses, insects, and mammals) [22]. The plant material itself, whether rooted or unrooted, also plays a role in the success of plants in the establishment phase. For example, rooted poplar stem cuttings have 30% higher survival rates than unrooted cuttings [23,24]. Since rooted cuttings are, not only more expensive, but also more difficult to plant, most tree nurseries in Germany only provide unrooted poplar stem cuttings [25]. Black locust, on the other side, can only be used for plantations as rooted cuttings [16,25]. Another source of variation in plant survival following plantation is the genotype. For example, Schirmer and Haikali [26] had shown that standard national cultivars of poplar such as Max 1 and Hybride 275 had a higher survival rate (85%) than AF2 (71%). A low mortality of "Max"-cultivars was confirmed in another study [27].

The ability of different poplar genotypes to grow in low-input short rotation coppices needs to be assessed in two steps. First, their survival rates under varying site conditions have to be tested. Second, their growth performance needs to be analyzed under different site conditions.

Unfortunately, not much is known about the interactions between poplar and black locust when grown in mixture. This is particularly true when it comes to stand establishment and thus survival. It is not clear yet, whether, at some point in stand development, the expected beneficial effects of admixed *Robinia* on poplar do, in fact, materialize.

By examining the survival performance of eight different poplar genotypes in the establishment phase of pure and mixed short rotation coppices with black locust, we hypothesized (1) that mortality rates differ between different poplar genotypes, and (2) that the mortality of poplar in mixture with black locust is lower than in pure stands.

2. Materials and Methods

2.1. Study Sites and Species

Two plantations were established in April 2014 at the research farms of the Georg-August-University Göttingen in Deppoldshausen (51.581° N/9.967° E) and Reinshof (51.484° N/9.923° E), Germany. Göttingen has a warm temperate climate, without a dry season. According to DWD Climate Data Center (CDC) [28] the mean temperature in Göttingen for the last 30 years (1988–2018) was 8.7 °C and mean rainfall 645 mm per year. During the study period, between 2014 and 2016, rainfall was distributed very unevenly throughout the vegetation period (Figure 1).

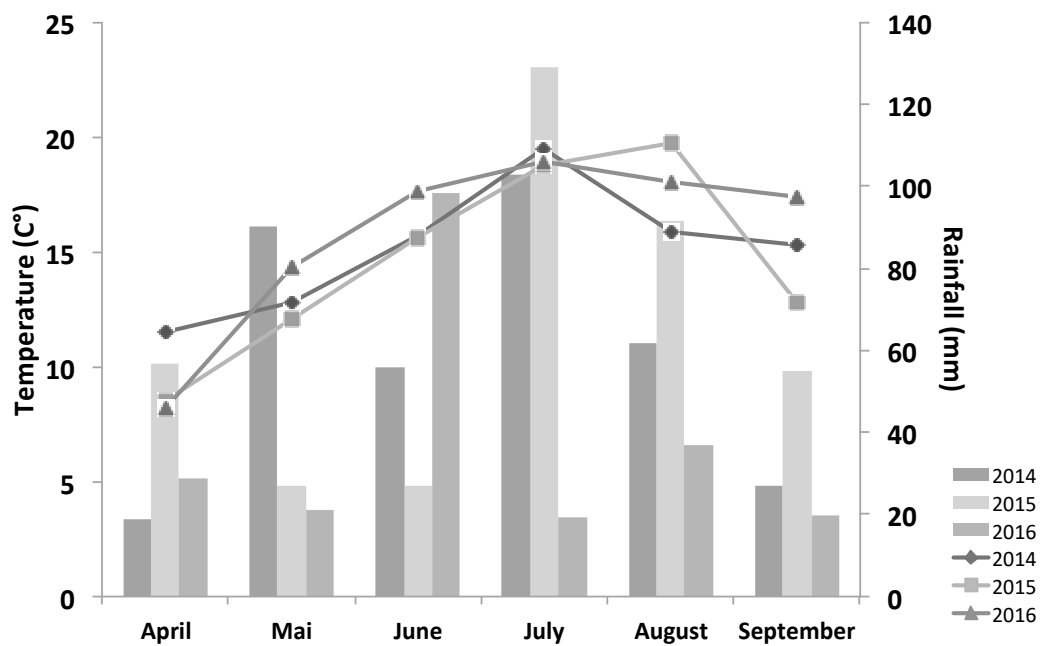


Figure 1. Climatograph for Göttingen, Germany. The columns indicate the total rainfall (mm) and the lines the average temperature (°C) for each month in the growing season during the study period (2014–2016). Data source: DWD Climate Data Center [28].

Eight commercially used poplar genotypes representing different hybrids of *Populus deltoides* W.BARTRAM EX MARSHALL, *Populus maximowiczii* A.HENRY, *Populus nigra* L., and *Populus trichocarpa* TORR. & A.GRAY EX HOOK. were selected as test species (Table 1). Three black locust provenances were selected as mixture species (Northern German lowlands, HKG81901; Germany, HKG81902; Hungary, Nagybudmry).

Table 1. Genotypes and their parentage used in the experiment ($n = 512$ per genotype).

Name of the Genotype	Parentage
AF2	<i>P. deltoides</i> × <i>P. nigra</i>
Fritzi Pauley	<i>P. trichocarpa</i>
Hybride 275	<i>P. maximowiczii</i> × <i>P. trichocarpa</i>
I214	<i>P. deltoides</i> × <i>P. nigra</i>
Matrix 11	<i>P. maximowiczii</i> × <i>P. trichocarpa</i>
Matrix 49	<i>P. maximowiczii</i> × <i>P. trichocarpa</i>
Max 1	<i>P. nigra</i> × <i>P. maximowiczii</i>
Muhle Larsen	<i>P. trichocarpa</i>

The site at Deppoldshausen is situated on marginal land. The shallow (<60 cm deep) and stony soil is classified as Calcaric Leptosol, according to the FAO classification system, and is characterized by its low ability to hold water. In Deppoldshausen (from here on referred to as ‘marginal site’), rainfall and annual mean temperature were lower than in Reinshof (by 50 mm yr^{-1} and -0.6 °C) during the observation period (2015–2016). Reinshof’s (from here on referred to as ‘fertile site’) soil is classified as Gleyic Fluvisol, a young fertile soil with high water storage capacity.

Four blocks were established at each site. Each block was comprised of 40 plots representing either pure (*Populus* genotype or *Robinia* provenance) or mixed stands (alternately planted *Robinia* and *Populus*). The following stand types were established on each block: 8 poplar plots (1 plot per genotype), 24 mixed plots (= 8 poplar genotypes × 3 *Robinia* provenances), 8 *Robinia* plots (4 plots “HKG81901”, 2 plots “HKG81902”, 2 plots “Nagybudmry”). Twenty-five trees were planted per plot in a $1 \times 1 \text{ m}$ spacing resulting in a total of 8000 trees (2 sites × 4 blocks × 40 plots × 25 trees). Mixed

plots comprised 13 poplar and 12 black locust trees. Following standard practice in short rotation forestry and similar experiments [16] unrooted poplar stem cuttings (25 cm in length) and rooted black locust nurslings were hand planted. All plots (mixed and pure) planted with the black locust provenance "Nagybudmry" were excluded from the final analysis, due to low quality of the nurslings and consequent high mortality of this provenance (>50%).

The plantation was treated as a low-input system, i.e., no fertilizers or herbicides were applied. Instead, ground vegetation was mown on both sites once in June 2015 and twice in the summer of 2016. Due to severe drought, the trees at the marginal site were irrigated two times in the first two weeks immediately after planting in 2014. There was no irrigation at any time at the fertile site. Vole control was carried out twice a year in the first two years at both sites after planting by application of rodenticide to minimize rodent damage. Since the two sites differ in soil fertility, regional precipitation, temperature, and irrigation, no separate analyses were carried out. Instead, we explored whether the poplar genotypes and the mixture types differed in seedling survival across sites.

2.2. Survival and Competition Survey

An on-site survival survey was done twice a year between 2014 and 2016, at the beginning and at the end of each growing season. Each plant was surveyed six times, registering its condition (living or dead). From these data, mortality was calculated on two levels. First, we calculated mortality after 1, 2, and 3 years on the single tree level as the ratio of dead tree individuals of a given genotype and the total tree number at the start of the experiment. Second, we calculated mortality on the plot level, i.e., mortality was defined as mean value of the plot wise ratio of dead tree individuals of a given genotype and the total tree number at the start of the experiment.

In order to quantify and evaluate the effect of competition on tree survival on a single tree basis, we calculated the Hegyi competition index (HgCI) [29] based on tree data collected in winter 2015/2016 (prior to the growing season of 2016). This individual tree competition index is distance dependent and requires a prior selection of competitors [30]. The selection was made by choosing all directly neighboring trees, which—taking into consideration the arrangement of the planted trees—were located within a radius of 1.4 m. The index was calculated as: $HgCI_i = \sum_{j=1}^n \frac{d_j}{d_i} \frac{1}{dist_{ij}}$, where HgCI_i is the competition index according to Hegyi [29] for subject tree *i*, *d_j* is the root collar diameter (3 cm above ground) for competitor *j* (mm), *d_i* is the root collar diameter for subject tree *i* (mm), *dist_{ij}* is the distance between competitor *j* and subject tree *i*, and *n* is the number of competitor trees. The higher the HgCI value of a tree, the higher the competition it experiences from its neighboring trees.

2.3. Statistical Data Analyses

Mortality of poplars was analyzed at both the single tree and plot levels (see above). For the survival analysis on a single tree level, we conducted a Kaplan–Meier Analysis to build the standard survival object and produce estimates for the probability of survival in dependence of competition pressure. In a second step, we fitted a Cox proportional hazards model by making use of the covariates 'genotype' and 'site' to test for model significance. The survival analysis was based on the R package 'survival' [31] and the visualization was based on the R package "ggplot2" [32]. For analyzing mortality on the plot level we used genotypes, stand types and sites as explanatory variables, which were statistically analyzed using the R software, version 3.5.1 (R Development Core Team, 2018, Vienna, Austria). Generalized linear models with binomial error structure were used to quantify the effect of explanatory variables on plot level mortality, which was described as proportion data (deceased trees/survivors) in the models. In the case of overdispersion, we used a quasibinomial error structure [33]. Differences between genotypes, between stand types, and between sites were analyzed using the *glht* function of the "multcomp" package [34] with Tukey contrasts.

3. Results

3.1. Mortality of Monocultures and Mixtures across Genotypes

After three years, an overall of 32% of all poplar trees in the mixed stands had died, while the corresponding value of the monocultures across genotypes was only 26%. Interestingly, in the first two years after planting (2014 and 2015) poplar mortality was not significantly different between pure and mixed stands. In the third year, however, there was significantly higher mortality in the mixtures. In 2016, pure stands lost an average of 5% of their poplar trees, while mixed stands lost 9% (Figure 2). The mortality of black locust after three years was 14% in both monoculture and mixture.

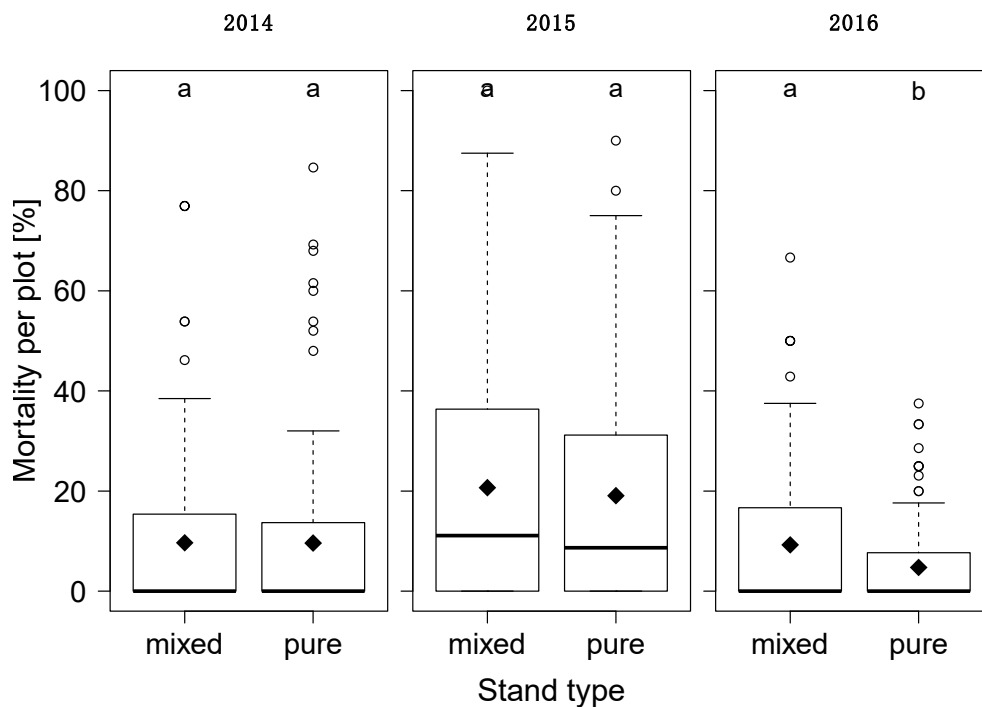


Figure 2. Poplar mortality across sites for mixed and pure stands in the first three years after planting. Mean values are marked by \blacklozenge . Different letters (a, b) indicate significant differences between the stand types at significance level $p < 0.05$.

3.2. Mortality of the Different Poplar Genotypes

Mortality proportion of the genotypes for the entire period of three years after planting was determined for each plot. Observed variation in mortality was then used to test the survival in pure stands on the basis of genotype (Figure 3), in order to exclude the impact on mortality found in mixtures in the third year (Figure 2). Significant differences between the genotypes were observed (Figure 3 and Table 2): Max 1 proved to be the genotype with the lowest mortality in this experiment with a mean mortality rate of 5% per plot, followed by Hybride 275 (mean mortality rate of 14%). Matrix 11 and Matrix 49 also had mean mortality rates of <25%, while survival was rather low for AF2, Fritz Pauley, and Muhle Larsen (mean mortality rate of around 50%, Figures 3 and 4).

3.3. Competition Induced Mortality

In the third year (2016) survival of poplar in mixtures was significantly lower than in pure stands (Figure 2). The mean mortality rate nearly doubled in mixtures, from 5% in pure stands to 9% in mixtures. The differences in mortality were paralleled by differences in individual tree competition quantified by the Hegyi-index at the end of the second year (Figure 5), suggesting that interspecific competition was higher than intraspecific interference.

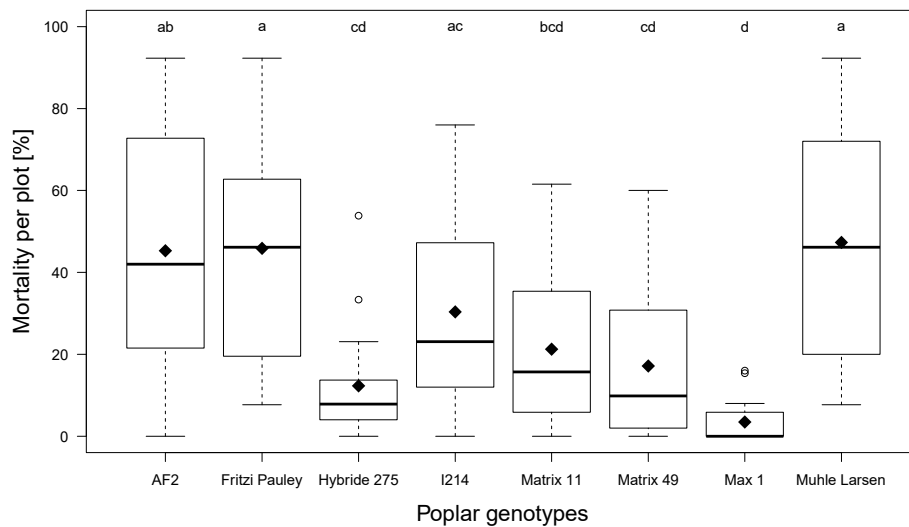


Figure 3. Mortality of all eight different poplar genotypes in pure stands, for the whole period of 3 years after planting across both study sites on the plot level. Mean values are marked by \blacklozenge . Different letters (a, b, c, d) indicate significant differences between the genotypes at significance level $p < 0.05$.

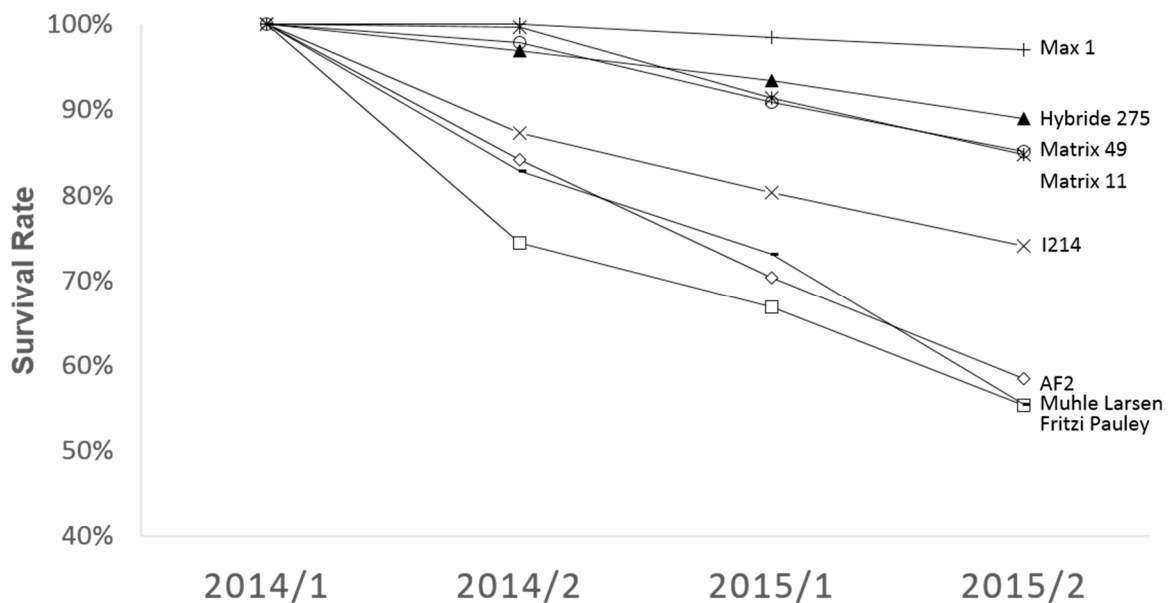


Figure 4. Survival rates of eight genotypes across sites and stand types on the single tree level in the first two years after planting. The survival rate was calculated as the ratio of living poplar plants of the respective genotype at a given time to the total number of planted trees of that genotype. The survey was done before (/1) and after (/2) the vegetation period in 2014 and 2015.

The Kaplan–Meier survival analysis resulted in a significant decrease in poplar survival probability with increasing competition (Figure 6). In the case of high competition values ($HgCI > 40$), the number of observations was very small, which resulted in very wide confidence intervals. The Cox proportional hazards model, including genotype and site as covariates, was significant for all three overall tests (Likelihood ratio test, Wald test, Score logrank test) at the level of $p < 0.0001$.

Table 2. Analyses of variance (ANOVA), mean, and standard deviation at plot level for mortality proportion of all poplar genotypes. The letters represent the results of Tukey post-hoc comparisons between genotypes means (p -value < 0.05).

	Stand Type	AF2	Fritzi Pauley	Hybride 275	I214	Matrix 11	Matrix 49	Max 1	Muhle Larsen
2014	mixed	0.17 ± 0.18 ab	0.26 ± 0.27 b	0.052 ± 0.094 ac	0.10 ± 0.13 bc	0.00 ± 0.00 bc	0.029 ± 0.084 c	0.00 ± 0.00 bc	0.15 ± 0.10 bc
	pure	0.14 ± 0.15 bc	0.24 ± 0.24 b	0.022 ± 0.061 c	0.14 ± 0.18 bc	0.073 ± 0.021 ac	0.017 ± 0.032 c	0.00 ± 0.00 bc	0.20 ± 0.21 ab
2015	mixed	0.34 ± 0.24 ab	0.33 ± 0.32 ab	0.085 ± 0.17 bc	0.17 ± 0.19 ac	0.14 ± 0.17 bc	0.14 ± 0.13 bc	0.034 ± 0.056 c	0.41 ± 0.19 a
	pure	0.36 ± 0.28 a	0.31 ± 0.23 a	0.091 ± 0.13 b	0.14 ± 0.15 ab	0.16 ± 0.16 ab	0.13 ± 0.16 ab	0.025 ± 0.035 b	0.31 ± 0.22 a
2016	mixed	0.10 ± 0.13 ab	0.11 ± 0.15 ab	0.043 ± 0.069 ab	0.13 ± 0.15 ab	0.066 ± 0.087 ab	0.063 ± 0.093 ab	0.032 ± 0.054 b	0.19 ± 0.17 a
	pure	0.056 ± 0.092 ab	0.025 ± 0.047 ab	0.014 ± 0.031 ab	0.071 ± 0.096 ab	0.069 ± 0.11 ab	0.042 ± 0.063 ab	0.011 ± 0.029 b	0.091 ± 0.11 a

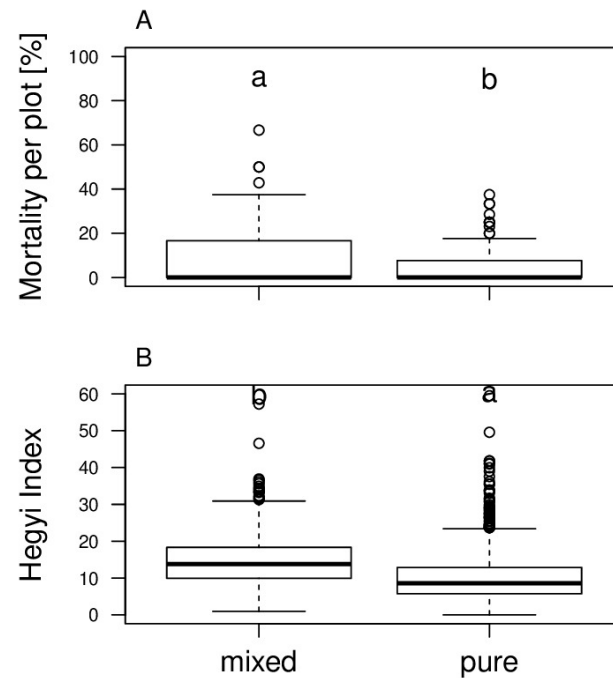


Figure 5. Mortality of poplar for the third year (2016) on the plot level for both mixed and pure stand types (A) and competition values (Hegyi.index) of poplar on individual tree level for both mixed and pure stands at the end of the second year (2015) (B). Solid lines in the boxes represent the median, the bottom and the top of the box represent the first and third quartile (IQR). Each whisker represents 1.5 IQR. Different letters (a, b) indicate significant differences between stand types, p < 0.05.

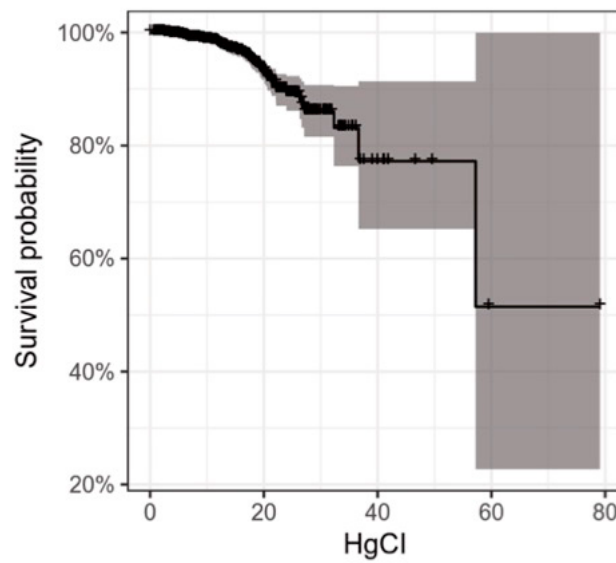


Figure 6. Graphic summary of the Kaplan–Meier analysis showing the competitive pressure as Hegyi competition index (HgCI) on the *x*-axis and the probability of survival on the *y*-axis for the third year after planting.

3.4. Growth Performance of Poplar in Pure and Mixed Stands

The faster growth of *Robinia* was significant over the entire period of the study (Figure 7). Between the analyzed provenances "HKG81901" and "HKG81902", there were no significant differences regarding growth. This trend was independent from stand type, i.e., poplar growing in pure stands yielded lower basal area per tree than black locust trees in pure stands. The same was true for mixed stands (Figure 7). No significant difference in basal area was observed between poplar growing in pure stands and mixtures, indicating that the mixture of *Robinia* did not increase poplar growth at this stage (Figure 7). Black locust, on the other hand, has a significantly higher growth in the mixtures, which indicates a benefit, in terms of growth, from mixed coppice with poplar (Figure 7).

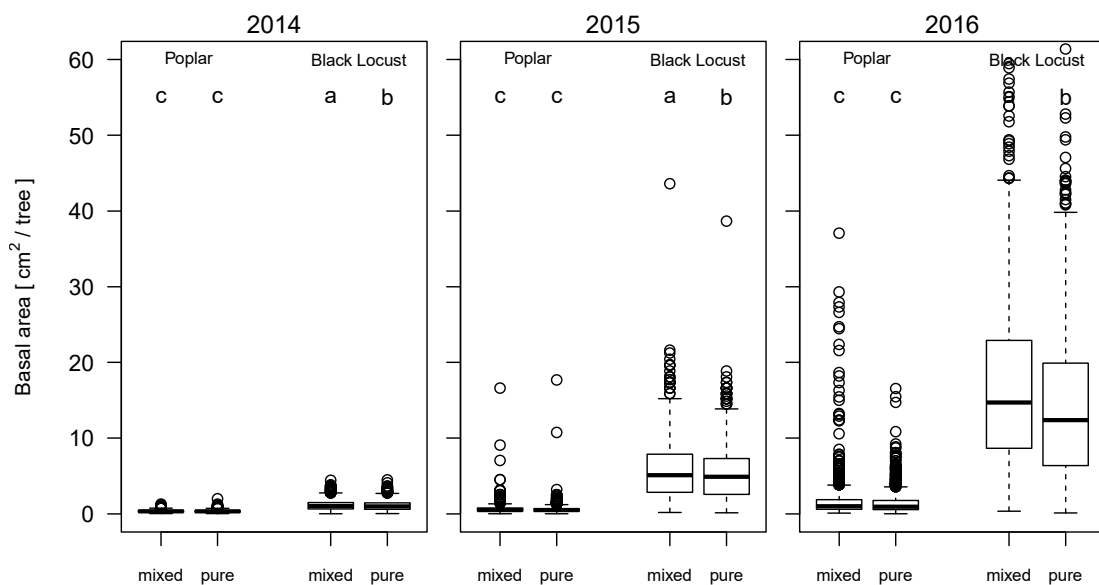


Figure 7. Basal area of poplar and black locust for the three years of the study in mixed and pure stands. Solid lines in the boxes represent the median and the bottom and the top of the box represent the first and third quartile (IQR). Each whisker represents 1.5 IQR. Different letters (a, b, and c) indicate significant differences between species and stand types, *p* < 0.05.

4. Discussion

Through selective breeding, interspecific hybridization, and cloning, poplar has been genetically improved to enhance its efficiency in intensive monocultures [35]. The optimization of poplar for short rotation coppicing has been the subject of many studies over the past decades [36–39]. In Germany, the well-adapted genotypes, e.g., Max 1 and Hybride 275, are regularly used for the production of biomass, and new genotypes have also been developed and tested for better growth efficiency [26,38]. However, quantitative studies of the performance of poplar genotypes in mixed short rotation, more precisely in mixtures with black locust, are lacking. Rédei et al. [17] and Oliveira et al. [16] observed that planting white poplar (*Populus alba*) and black locust in mixed stands may lead to increased yield. Our findings, however, demonstrate that young poplars can also respond to black locust neighbors with higher mortality. In our study, black locust dominated the mixed stands and doubled poplar mortality after the establishment period (Figure 2, Figure 5, Figure 7, and Table 2).

4.1. Genotypical Variation

We analyzed poplar mortality of eight different poplar genotypes in pure stands in the first three years after planting, and we hypothesized that the poplar genotypes differ in their survival ability. Our experiment showed that mortality strongly differed between the genotypes. Max 1 and Hybride 275 had the lowest mortality rates (Figures 3 and 4), demonstrating the capacity of these genotypes to succeed under a short rotation coppice regime. These two genotypes are approved and recommended in Germany standard varieties that usually have high survival rates [26]. Fritzi Pauley, AF2, and Muhle Larsen had the highest mortality rates of more than 40% (Figure 5). Such low survival capacity make the genotypes unsuitable for short rotation coppices, since the remaining plants cannot compensate for the missing biomass [26]. However, in past experiments, the genotypes Matrix 49, Matrix 11, Fritzi Pauley, and AF2 produced good to very good results (> 70% survival rate) and were thus recommended in a recent study [26].

In our experiment, genotypical variation was most obvious in the first two years. In the third year, mortality rates dropped and variation between genotypes declined (Table 2). According to Melkinova et al. [40], most studies report that male *Populus* plants have higher tolerance to environmental stresses such as drought than female plants. However, our findings do not support these studies. In our experiment, the male poplar genotype (AF2) did not as good as the female genotype Max 1, but showed no significant differences between the other three female genotypes (Fritzi Pauley, I214, Muhle Larsen). Our data shows no clear evidence that the differences in survival between genotypes could be explained by sex.

A recent study [41] shows that phenotypic plasticity may explain, to a certain extent, drought tolerance of *Populus nigra*. Less plastic genotypes were more drought tolerant than genotypes with high plasticity. Another study on comparative transcriptomic analysis reports "divergent gene expression patterns among the genotypes in response to drought" [42]. Based on our research, we can only speculate whether differences in gene regulation or plasticity and hence different strategies to deal with environmental stress (avoidance versus tolerance) were also the reason for the different performance of genotypes of our study.

4.2. Mixing Effects on Mortality

In our second hypothesis, we stated that the mortality of poplar in mixture with black locust is lower than in pure stands. This hypothesis was clearly disproved. In the initial phase (2014 and 2015), survival of the genotypes was not significantly different between pure and mixed stands (Figure 2). This finding indicates that mortality during the phase of establishment was mainly driven by the environmental conditions after planting [19] and not by intertree competition. Besides drought stress, rodent damage and grass competition may have also been important mortality factors in the first two years [38,43]. We assume that competition between trees was intensified in the third year after

planting—as tree crowns started overlapping and roots extended into each other’s rooting zones, limiting sunlight, soil nutrients, and water. Furthermore, at this point, it is likely that the increasing crown cover also led to less weed competition. At the same time, a decline in ground vegetation biomass may have lowered rodent abundance [22,44]. Therefore, we speculate that in the third year, damage by rodents and weed competition impacted poplar survival to a lower degree than in the first two years [23]. Hegyi’s competition index demonstrated the intensified competition of black locust over poplar in mixtures. At both sites, HgCI was significantly higher in mixtures than in pure stands (Figure 5B). Accordingly, the single tree survival analysis showed a significant decrease in the probability of survival of poplar with increasing competition (Figure 6).

Our main finding in the third year, that the presence of black locust may have resulted in poplar mortality, is in sharp contrast to the study of Oliveira et al. [16], who observed that competition between *Populus alba* and *Robinia pseudoacacia* had no influence on their mortality rates. It seems as if site fertility plays a major role. On fertile sites such as ours, where black locust grows much more rapidly than poplar, delaying the planting of N-fixing species into a coppice for a few years may prevent high mortality of the latter. It is well known from other studies that this strategy can help to balance the growth of the two species. For example, Radosevich et al. [45] found that red alder (*Alnus rubra*), an N-fixing species, overgrew Douglas fir seedlings (*Pseudotsuga menziesii*) when planted at the same time under beneficial site conditions, but not if red alder were planted some years after Douglas fir. Interestingly, no such delayed plantings were needed on less fertile sites, because at those sites, Douglas fir could keep up with alder [45]. A delayed planting of *Robinia* is also suggested by the finding that poplar did not achieve better growth in mixed stands than in pure stands (Figure 7). Thus, the losses in stem numbers of poplar in the mixed stands could not be compensated or even over-compensated by a potentially better growth performance due to the nitrogen input by *Robinia*. This finding also points towards the assumption that our sites were too fertile for showing a positive mixing effect by *Robinia*. Comparable results have been achieved by Forrester et al. [46]. In their study, a positive effect of the admixture of the N-fixing species *Acacia mearnsii* on the growth of *Eucalyptus globulus* was not found on soils that were rich in nitrogen, whereas it was reported on poor sites.

5. Conclusions

In our experiment, poplar genotypes Max 1 and Hybride 275 proved to be the best poplar genotypes with respect to survival capability. AF2, Fritzi Pauley, and Muhle Larsen had very low survival rates and proved to be unsuitable for our sites. The admixed black locust reduced poplar survival across all genotypes. The expected advantage of mixing an N-fixing tree with an N demanding tree like poplar on growth was not observed. Instead, even if they had occurred, they were overshadowed by black locust’s dominance and competitiveness.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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