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Effects of Reforestation and Site Preparation Methods on Early Growth and Survival of Scots Pine (*Pinus sylvestris* L.) in South-Eastern Poland

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Abstract: Successful tree regeneration is a key process in ensuring forest sustainability and one of the most crucial investments made in silviculture. This study compared the effects of three reforestation methods (planting, direct seeding, and natural regeneration) and three mechanical site preparation methods (double mould-board forest plough (FP); active plough (AP); and forest mill (FM)) on biometric parameters, survival, and density of Scots pine (*Pinus sylvestris* L.) seedlings in the first 4 years of growth in a clear-cut area in south-eastern Poland. Planted seedlings were higher, thicker in root collar, and had higher survival rates after the fourth growing season than trees from natural regeneration and direct seeding. Site preparation methods did not affect the density of planted seedlings. After natural regeneration and direct seeding, seedling density was lower and less homogeneous (plots with no seedlings) in FM soil preparation in comparison to other methods. The survival of pines in all reforestation methods was not affected significantly by site preparation methods. Our results indicate that the best mechanical site preparation method for planting is FM, as this is the one that least disturbs the soil environment. For direct seeding the best results were achieved after AP preparation. Natural regeneration of Scots pine was most effective after FP use, and in relatively wet years also after AP use.

Keywords: artificial regeneration; direct seeding; natural regeneration; planting; soil preparation

1. Introduction

Scots pine (*Pinus sylvestris* L.) is the most widely distributed conifer species in the northern hemisphere and is the most important forest forming species throughout East-Central Europe [1]. Scots pine forest stands can be regenerated in three ways: planting, direct seeding, and natural regeneration. In the Polish forestry model, planting is the most commonly used method. Usually 8000–10,000 one-year-old seedlings are planted per hectare [2]. In recent years, natural regeneration of forest stands, by self-sown seedlings, has been practiced more frequently. In 2014, natural regeneration of all tree species accounted for 16.3 per cent of the total reforested area. A further increase of the percentage contribution of natural regeneration in Poland is possible primarily thanks to introduction of natural regeneration of Scots pine, which is the major tree species in the country, constituting 58.1 per cent of its total forest area [3]. Natural regeneration of pine is facilitated by natural conditions (prevalence of coniferous forest sites, frequently high pine seed yields) and promotion of this reforestation method, given that it is more ecologically and economically favourable than the others [4,5].

Direct seeding is an old reforestation method, currently rarely used. In Poland in the 1960s and 1970s, 5.3–7.8 per cent of clear-cut areas were reforested in this way, compared to 2.6 per cent in the 1980s [6,7]. This method has many advantages. First, the costs of reforestation are lower than those of planting [8]. Second, direct seeding imitates natural regeneration. Third, forest stands formed in this way, growing in high densities are characterized by high timber quality [7]. The main reasons why direct seeding is now so rare is the risk of unsuccessful regeneration because of the greater influence of biotic and abiotic stress factors on germinating seeds than on planted seedlings [9–11]. Comparative studies show that, if regenerated by direct seeding or self-sowing, Scots pine and other tree species grow more slowly and are less likely to survive than planted seedlings [9,12,13].

To create optimum conditions for seed germination and seedling growth in a clear-cut area, mechanical site preparation (MSP) is recommended [2,14], primarily to limit competition with other plants for light, water and nutrients [15] and decreases damage caused by the pine weevil (*Hylobius abietis* L.) [16]. Allelopathic effects in the ground layer of vegetation also may limit seed germination and seedling growth [17,18]. MSP can modify the physical properties of soil, such as water content, aeration, temperature and bulk density, as well as its chemical properties, such as organic matter content, nutrient availability and pH [19–22]. Furthermore, most site preparation methods can cause soil erosion if not carefully implemented and adapted to the specific site characteristics and climate [23,24]. For example, in steep forest-lands in northern Spain, mechanized forest operations including down-slope ploughing significantly increased soil losses, with effects on site productivity [25].

The impact of MSP on seed germination and seedling growth and survival may vary depending on climatic conditions, site types and tree species [9,14,21,26,27]. Basic MSP methods involve one or more of the following: removing the forest floor to expose mineral soil, inverting forest floor and mineral soil, elevating the mineral soil and mixing forest floor with mineral soil [21,28]. Various tools and machines can be used for MSP that differ in the degree of soil disturbance, as measured by the area and depth of the disturbed soil [29,30]. According to many comparative studies, more intensive scarification methods usually result in a higher initial number of seedlings and better subsequent growth and survival [31–34]. Results of the studies conducted so far show that, on longer time scales, tree growth is poorest after removal of the forest floor to expose mineral soil, whereas it is best after mixing the forest floor with mineral soil [21,35].

In Polish forestry, for many years the most commonly used tool for MSP in clear-cut areas was the double mould-board forest plough (FP). FP severely disturbs the soil environment, affecting the entire clear-cut area. In recent years, two other methods have also been widely used: active plough (AP) and forest mill (FM). Their major advantage is that they affect the soil environment to a much smaller extent [30]. Plantations resulting from planting and self-sowing on soils prepared by FM are characterized by low density and survival rate but very good growth rate compared with other MSP methods [36–38].

This study aimed to compare the effects of three MSP methods (FP, AP, FM) and three reforestation methods (planting, direct seeding, natural regeneration) on growth, survival and density of Scots pine in the first four growing seasons in a clear-cut area.

We hypothesize that (i) Planted seedlings of Scots pine would growth faster and have a higher survival rate that ones regenerated by direct seeding or self-sowing methods; (ii) FP site preparation method would favour more natural regeneration in terms of density and survival rate than AP and FM; (iii) All seedlings in FM treatment would growth faster than in treatments with soil scarification made by FP and AP.

Our results are intended to assist in the choice of the best MSP method depending on the selected reforestation method.

2. Materials and Methods

2.1. Study Area

Field research was conducted in Narol Forest District, located 300 km south-east of Warsaw, Poland (50°20′ N, 23°20′ E; WGS 84). The soil in the study area was classified as brown podzolic, formed on loose sands with typical mor humus. Before tree felling, the forest stand was composed of 87-year-old Scots pine (*Pinus sylvestris* L.) (stand volume ca. 240 m^3 ha^{-1}; stand density ca. 430 stems ha^{-1}; average tree height ca. 22 m). The herb layer was dominated by *Calluna vulgaris* (L.) Hull, *Vaccinium myrtillus* L., *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Festuca ovina* L. The plant community was classified as *Peucedano-Pinetum* W.Mat. [39]. The trees were felled in November and December 2007, and the soil was prepared in February 2008. The study site (40 m × 135 m) was located in the clear-cut area. Its axis was oriented in the SE–NW direction. In the south-western part, the site borders an 88-year-old pine forest. On the other sides, the site borders clear-cut areas planted with 1-year-old Scots pine.

2.2. Weather Conditions

The source of data on monthly precipitation and mean air temperature during the experiment (2008–2011) was the weather station in Horyniec Zdrój, situated ca. 20 km from the study site. To illustrate the moisture conditions more clearly, we calculated the de Martonne aridity index (AI) [40] for each month of the growing season by using the formula:

$$\mathrm{AI} = 12P/(T + 10) \quad (1)$$

where P is the monthly precipitation (mm) and T is the monthly mean air temperature (°C). According to the World Meteorological Organization [41] AI indicates the months when irrigation is necessary. Generally, irrigation is necessary when AI < 20 (Table 1).

Table 1. Monthly average air temperature (°C), monthly precipitation (mm) and de Martonne aridity index during the 2008–2011 growing seasons at the Horyniec Zdrój weather station.

Month	Temperature (°C)				Precipitation (mm)				Aridity Index			
	2008	2009	2010	2011	2008	2009	2010	2011	2008	2009	2010	2011
April	8.9	10.5	8.9	9.8	60	8	27	38	38.1	4.7	17.1	23
May	12.9	12.7	14.3	13.5	94	94	122	34	49.3	49.7	60.2	17.4
June	17.2	16.1	17.7	17.6	39	82	72	62	17.2	37.7	31.2	27
July	17.7	19	20	18.2	104	33	109	141	45.1	13.7	43.6	60
August	18.3	17.4	19	18	82	40	74	49	34.8	17.5	30.2	21
September	12.3	14.1	11	14	95	32	96	23	51.1	15.9	54.9	11.5
October	9.7	7.6	4.5	6.6	42	91	13	21	25.6	62	10.8	25.2

2.3. Experimental Design and Treatments

The experiment followed a two-factor block design consisted of nine variants distinguished on the basis of MSP (FP, AP, FM) and reforestation (planting, direct seeding, natural regeneration) methods randomly assigned to each of the nine blocks. Each block was ca. 4.5 m wide and 135 m long and was divided into nine plots (4.5 m × 15 m). To take into account a spatial environmental variation, blocks were located parallel to the stand border (Figure 1).

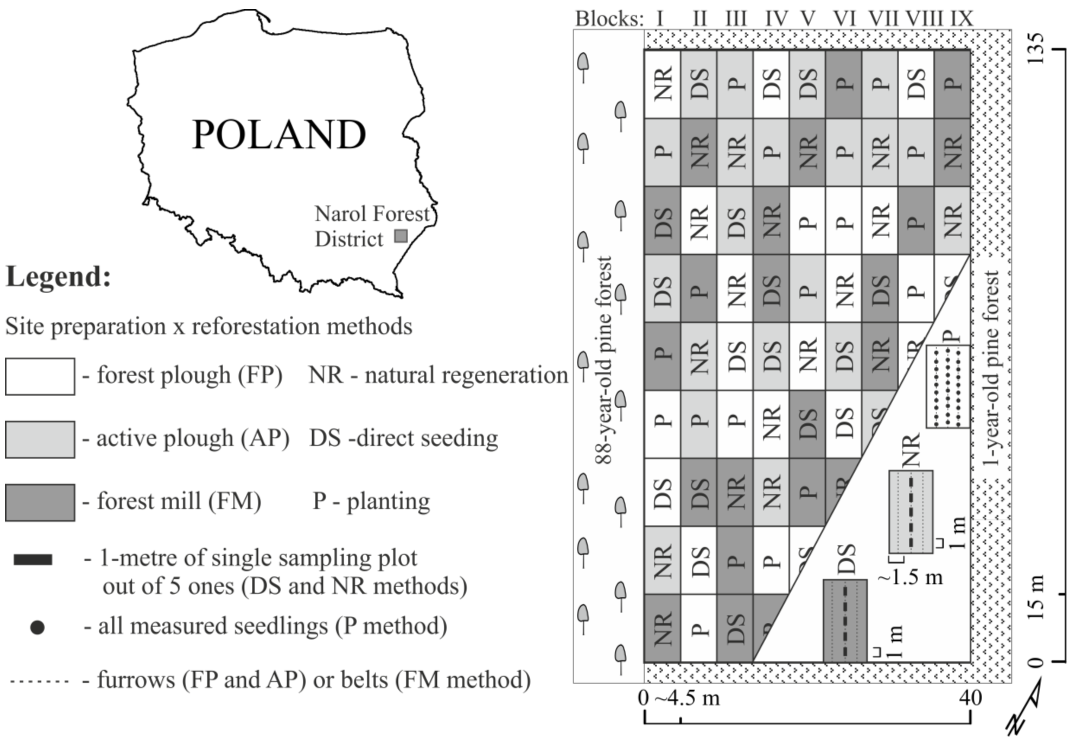

Figure 1. Study location and experimental design.

MSP was performed with three tools: LPz OTL double mould-board forest plough (FP), P1T active single-disc plough (AP) and FL forest mill (FM) manufactured by Ośrodek Techniki Leśnej, Jarocin, Poland (http://www.otljarocin.lasy.gov.pl/preparation-of-soil-and-afforestation).

The furrows made by FP are rectangular, 70 cm wide and 5–10 cm deep. Leaf litter and the humus layer are cut and placed as ridges on both sides of the furrow, exposing mineral soil in the furrow. Furrows account for 50 per cent of the surface area, and the other 50 per cent is covered by ridges. In AP, the rotating disc makes the furrow parabolic, 40 cm wide and up to 10 cm deep. Leaf litter and a portion of the humus are partly mixed and placed on the ridge. The bottom of the furrow is scarified and covered with a mixture of humus and mineral soil. In this MSP method, furrows account for 40 per cent of the surface area, ridges for 40 per cent and undisturbed soil for the remaining 20 per cent. The working part of FM is a horizontal cylinder with cutting blades, revolving at a rate of 1000 rpm. FM grinds and mixes the forest vegetation, leaf litter, humus and mineral soil to a depth of 30 cm in belts 40 cm wide (27 per cent of surface area). The undisturbed belts between them are 110 cm wide (73 per cent of surface area). The distance between centres of neighbouring furrows or belts in all the MSP methods is 1.5 m [30].

In the directly seeded variants in spring (15 April 2008), pine seeds were sown manually (20 per metre of row, seeds spaced 5 cm apart) in the furrows made by FP and AP and on the belts made by FM. The seeds were next covered with a thin layer of sand (ca. 3 mm). At the same time, seeds were sown in a forest nursery located 10 km away from the study site to produce seedlings to be used for planting. The seeds were derived from the forest stand bordering on the study site, which was also the source of seeds for natural regeneration. The first self-sown seedlings in the plots for natural regeneration appeared at approximately 20 May 2008, ca. 10 days after the manually sown seedlings. Seedlings from the nursery were planted in the spring of 2009, in furrows and on belts, spaced 0.7 m × 1.5 m apart. Self-sown seedlings appearing on the plots used for direct seeding and planting were physically removed throughout the experiment. On the plots set aside for natural regeneration, the seedlings that germinated in the first year were marked. In the natural regeneration plots, seedlings that appeared in the second year were also included in the measurements, whereas the seedlings that germinated in the third and fourth years were removed. In the second year, sheep wool was used to protect apical buds of the seedlings against animal browsing and in addition experimental area was fenced in order to protect against herbivore damage. Weeds and natural regeneration of shrubs, as well as pioneer tree species were not abundant in the clear-cut area, so manual weeding and extraction of single seedlings of silver birch was sufficient, performed in July of the second and third years of the experiment.

2.4. Measurements

Individual seedlings were counted and measured four times, with seedling height and root collar diameter recorded in successive growing seasons. The first measurement was performed in spring (2009), before the growing season, after planting of the seedlings. Pine seedlings were then 1 year old in all the variants of the experiment, except of zero years old seedlings appeared in the second year. Successive measurements were taken in autumn (2009, 2010, 2011), after the growing season. In the plot with planted seedlings, all seedlings were measured. In the directly seeded and self-sown variants, sampling plots were established regularly. A single sampling plot in the directly seeded variant was a 1-m segment of a row, while in natural regeneration (self-sown), it comprised a 1-m segment of a furrow or belt across its whole width (0.7 m in FP, 0.4 m in AP and FM). For each plot, 5 sampling plots were established. In total, in the experiment focusing on natural regeneration and direct seeding and three MSP methods, 270($2 \times 3 \times 5 \times 9$) sampling plots were established (Figure 1). Seedlings density was calculated according to number of seedlings per measured area.

2.5. Statistical Analysis

The mean values for the different response variables for each block and treatment were calculated before the analyses were conducted. Before the further analyses, we tested the normality of data distribution in individual experimental variants (Shapiro–Wilk test) and homogeneity of their variance (Levene's test). For the variables that met these assumptions (seedling height, root collar diameter and density), 2-way analysis of variance (ANOVA) and the Tukey honest significant difference (HSD) post hoc test were performed ($\alpha = 0.05$). The general linear model (GLM) from R package 'nlme' [42] and the following Equation was applied:

$$Y_{ijm} = \mu + \alpha_i + \beta_j + \gamma_m + (\beta\gamma)_{jm} + \varepsilon_{ijm} \quad (2)$$

where, μ is the overall mean, α_i is the block effect (i = 1–9), β_j denotes the reforestation method effect (j = 1–3), γ_m is the MSP method effect (m = 1–3) and ε_{ijm} is the experimental error.

For survival rate (per cent), the nonparametric Kruskal–Wallis test ($\alpha = 0.05$) was used. Analysis of regression was performed to assess the relationships between root collar diameter and root collar diameter increment as well as between height and height increment. Finally, a logistic regression was applied to analyse the influence of biometric parameters, the MSP method and regeneration method on survival rate in the first years of growth. The statistical analyses were carried out using R 3.2.1 (R Core Team, 2016).

In the directly seeded and self-sown variants, for individual MSP methods (in per cent) we assessed evenness of seedling density in following classes: 0 = 'zero plots' (with no seedlings); 1 = 1–4 seedlings; 2 = 5–8 seedlings; 3 = 9–12 seedlings; 4 = 13–16 seedlings; 5 = 17–20 seedlings; 6 = 21–24 seedlings; and 7 = more than 25 seedlings.

In the natural regeneration plots, we calculated the relative abundance (per cent) of seedlings that emerged in 2008 and 2009 for individual MSP methods. We assumed that 100 per cent was the number of seedlings in FP plots, because this method is the oldest and the most common in practice.

3. Results

Throughout the experiment, seedling height depended on the reforestation method ($p < 0.0001$). In the first 3 years, 2-way ANOVA showed that this parameter is also affected by the MSP method

(p = 0.0446, p = 0.0078 and p = 0.0061, respectively, Supplementary Materials Table S1). During the 4 years of the experiment, planted seedlings were significantly taller than those in the other variants: after the second year nearly 100 per cent higher, after the third year 58.1 per cent and after the fourth year 67 per cent. The heights of seedlings from natural regeneration and direct seeding were similar (Figure 2). In the first 3 study years, seedlings growing in FM plots were significantly taller than those in FP plots, but in the first year this is true for direct seeding only. Seedling height correlated positively with annual height increment (Table S2, Supplementary Materials).

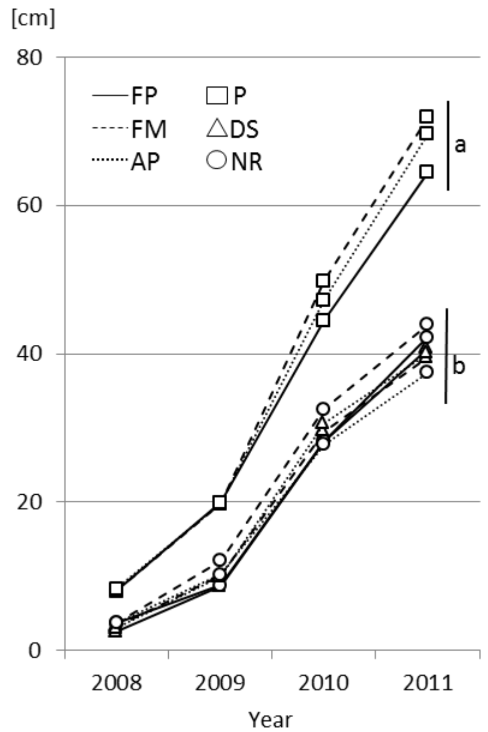

Figure 2. Mean height (cm) of Scots pine seedlings in 4 years for three reforestation methods (planting (P); direct seeding (DS); natural regeneration (NR)) and three mechanical site preparation methods (forest plough (FP); forest mill (FM); active plough (AP)). Different letters indicate significant differences in the Tukey HSD test, $p \leq 0.05$.

Root collar diameter changed in a similar pattern to that of seedling height. In the first 2 years of growth, this parameter was affected both by reforestation ($p < 0.0001$) and MSP methods ($p = 0.0006$ and $p = 0.0198$, respectively), but only by reforestation method ($p < 0.0001$) in the following 2 years (Table S1, Supplementary Materials). Planted seedlings were thicker than those from natural regeneration and direct seeding plots, 30.7 per cent thicker after 2 years, 48.4 per cent after 3 years and 62.4 per cent after 4 years (Figure 3). After the first year of growth, seedlings in FP plots were significantly thinner than those in FM plots. After the second year of growth, seedlings growing in FM plots were slightly thicker than the others. Root collar diameter correlated positively with annual diameter increment (Table S2, Supplementary Materials).

Mean density of seedlings depended both on reforestation and MSP methods (Table S1, Supplementary Materials). Seedling density was highest after direct seeding. In this reforestation method, differences in density depending on MSP method were significant during the 4 study years. Density in AP plots was significantly higher than in FM and comparable with density in FP plots. In the planted and self-sown plots (excluding self-sown seedlings that germinated in the second year), MSP variants did not differ significantly in density (Table 2).

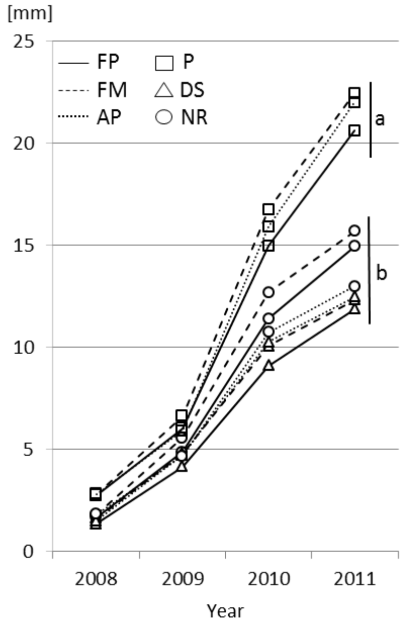

Figure 3. Mean root collar diameter (mm) of Scots pine seedlings in 4 years for three reforestation methods (planting (P); direct seeding (DS); natural regeneration (NR)) and three mechanical site preparation methods (forest plough (FP); forest mill (FM); active plough (AP)). Different letters indicate significant differences in the Tukey HSD test, $p \leq 0.05$.

Table 2. Average density (seedlings ha^{-1}, coefficient of variation in brackets) of Scots pine seedlings (excluding self-sown seedlings that germinated in the second year) in 4 years for three reforestation methods (planting, direct seeding, natural regeneration) and three mechanical site preparation methods (forest plough (FP); forest mill (FM); active plough (AP)).

Treatment	**Average Density (Seedlings ha^{-1}) in Year**			
	2008	**2009**	**2010**	**2011**
Planting FP	9651 (1.8) ^{c}	9602 (1.9) ^{c}	9536 (1.7) ^{c}	9536 (1.7) ^{c}
FM	9766 (0.9) ^{c}	9717 (1.1) ^{c}	9618 (1.6) ^{c}	9569 (2.2) ^{c}
AP	9668 (1.3) ^{c}	9634 (1.7) ^{c}	9420 (3.1) ^{c}	9371 (3.2) ^{c}
Direct seeding FP	61,809 (46.4) ^{a,b}	54,101 (54.9) ^{a,b}	50,396 (56.6) ^{a,b}	47,876 (59.6) ^{a,b}
FM	46,838 (26.8) ^{b}	42,243 (27.8) ^{b}	38,982 (28.2) ^{b}	36,907 (30.9) ^{b}
AP	70,850 (29.1) ^{a}	65,218 (31.2) ^{a}	60,771 (34.2) ^{a}	58,251 (39.9) a
Natural regeneration FP	11,265 (77.9) ^{c}	9783 (94.0) ^{c}	9190 (86.8) ^{c}	8449 (83.6) ^{c}
FM	6077 (58.2) ^{c}	4743 (61.5) ^{c}	4743 (61.5) ^{c}	4447 (63.6) ^{c}
AP	19,417 (73.9) ^{c}	16,008 (76.7) ^{c}	14,526 (66.3) ^{c}	13,488 (64.2) ^{c}

Different letters indicate significant differences in the Tukey HSD test, $p \leq 0.05$.

Seedling density in natural regeneration increased considerably in the second year as a result of germination of additional seedlings (Table 3). In 2008, the density of self-sown seedlings was highest in the AP plot, whereas in 2009, it was highest in the FP plot. Starting from the second year, density was significantly higher in FP plots (2009, 2010, 2011, $p < 0.0001$). After 4 years, the mean density (for all MSP methods collectively) in planted plots amounted to 9492 seedlings ha^{-1}, in the directly seeded plots, 47,678 seedlings ha^{-1} and in the naturally reforested plots, 8795 seedlings ha^{-1} when self-sown seedlings that appeared in the second year were excluded and 25,840 seedlings ha^{-1} with the additional seedlings.

Table 3. Relative abundance of Scots pine seedlings that germinated in the first and second growing seasons, seedling density (including self-sown seedlings that germinated in the second year) and survival rate of self-sown seedlings that germinated in the second year (mean and coefficient of variation in brackets) in natural regeneration, depending on mechanical site preparation methods (forest plough (FP); forest mill (FM); active plough (AP)).

Mechanical Site Preparation Method	Relative Abundance (%) of Seedlings That Emerged in Year		Density Including Self-Sown Seedlings That Germinated in Second Year (Seedlings ha^{-1}) [1]			Survival Rate (%) of Seedlings That Germinated in Second Year [2]	
	2008	2009	2009	2010	2011	2010	2011
FP	100	100	75,593 (58.2) ^{a}	62,550 (58.6) ^{a}	41,058 (60.8) ^{a}	81.1 (15.3) ^{a}	49.6 (37.8) ^{a}
FM	53	27	22,826 (68.7) ^{c}	18,528 (81.5) ^{c}	12,302 (93.2) ^{c}	76.2 (26.1) ^{a}	43.4 (34.8) ^{a}
AP	172	45	46,097 (86.7) ^{b}	37,648 (83.8) ^{b}	24,160 (82.0) ^{b}	76.9 (18.8) ^{a}	35.5 (63.3) ^{a}

[1] Different letters indicate significant differences in the Tukey HSD test, $p \leq 0.05$; [2] The same letters indicate no significant difference in Kruskal–Wallis test, $p \leq 0.05$.

The evenness of seedling density depended on reforestation (natural regeneration vs. seeding) and MSP methods (Figures S1 and S2, Supplementary Materials). The percentage contribution of sampling plots with no seedlings (zero plots) after the first year was on average 36.3 per cent in natural regeneration but on average only 3.7 per cent after seeding. In the second year of natural regeneration, the percentage of zero plots declined to 7.4 per cent as a result of the appearance of new self-sown seedlings. In FP plots with natural regeneration, from the second year until the end of the experiments, there were no sampling plots without seedlings. By contrast, after direct seeding, there were no zero plots in the first 2 years in the AP variant. The most favourable evenness of seedling density after 4 years of natural regeneration was observed in the FP variant, and after direct seeding, in the AP variant.

In the first year after direct seeding in the FM variant, seedlings developed from 35.0 per cent of sown seeds, compared to 46.5 per cent in FP and 53.0 per cent in AP plots.

Scots pine survival rate in the first 4 years of growth was affected only by reforestation method (H = 27.91 after the second, H = 30.10 after the third and H = 33.22 after the fourth year; $p < 0.0001$ in the Kruskal–Wallis test). The MSP method did not affect this variable (H = 0.2186, p = 0.8965 after the second; H = 0.4500, p = 0.7985 after the third; H = 0.0461, p = 0.9772 after the fourth year in the Kruskal–Wallis test). Planted seedlings were characterized by the highest survival rates: 99.6 per cent after 2 years, 98.3 per cent after 3 years and 98.1 per cent after 4 years. Significantly lower survival rates were recorded after direct seeding (88.2 per cent after the second, 81.2 per cent after the third and 76.5 per cent after the fourth year) and natural regeneration (82.7 per cent after the second, 79.7 per cent after the third and 75.9 per cent after the fourth year) (Figure 4). Survival rate (per cent) of self-sown seedlings in the second year reached 79.2 per cent after the third year of growth (2010) and 44.9 per cent after the fourth year (2011), and did not differ based on MSP method (after 3 years H = 1.18, p = 0.5544; after 4 years H = 2.81, p = 0.2450; Table 3).

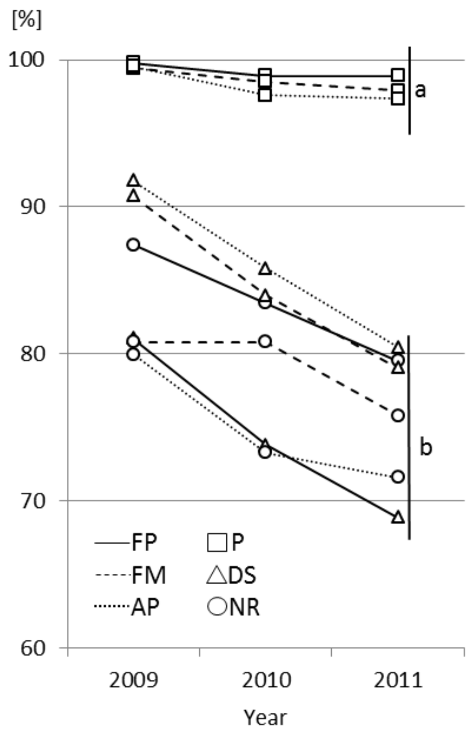

Figure 4. Mean survival (%) of Scots pine seedlings during 3 years for three reforestation methods (planting (P); direct seeding (DS); natural regeneration (NR)) and three mechanical site preparation methods (forest plough (FP); forest mill (FM); active plough (AP)). Different letters indicate significant differences in the Kruskal–Wallis test, $p > 0.05$.

Seedling survival, analysed by logistic regression, is related to biometric parameters and reforestation method. In successive years the relationships were as follows: in the second year survival rate depended on root collar diameter and reforestation method ($p < 0.0001$; $\chi^2 = 190.27$); in the third year, on seedling height and root collar diameter ($p < 0.0001$; $\chi^2 = 127.19$); and in the fourth year, on height and reforestation method ($p < 0.0001$; $\chi^2 = 125.30$). Higher survival rates were recorded for taller and thicker seedlings as well as for planted ones, compared to directly seeded and self-sown variants. Seedling density did not have any significant effect on survival rate in the period analysed.

4. Discussion

Reforestation (planting, direct seeding, natural regeneration) and MSP (FP, AP, FM) methods both significantly affected the analysed characteristics of forest regrowth: biometric parameters, survival rate and density of pine seedlings.

Our data on seedling height, showing that planted trees were 100 per cent taller after the second growing season and nearly 70 per cent taller after the fourth year, compared to seedlings from natural regeneration and direct seeding, are consistent with reports of other authors [9,13]. Similar differences were detected in root collar diameter: self-sown and directly seeded pine seedlings were thinner than planted ones. We also found that larger pine seedlings (taller and thicker in root collar) grew faster than smaller ones, as reported also by Collet and Moguedec [43]. This result indicates that planted seedlings can compete better for nutrients and other resources. It also suggests that planted pine seedlings can utilize improved soil conditions slightly better than seedlings from natural regeneration and direct seeding plots [44]. It can be also supposed that the better-developed foliage and better nutrient status at the time of planting of seedlings produced in a forest nursery stimulate higher rates of photosynthesis [45]. Another advantage of planted seedlings is that they more quickly reach a height of about 1.5 m, which reduces the risk of animal browsing, late spring frost or competition with herbaceous plants [29].

MSP method affected pine growth in height and root collar diameter in the first 2–3 years, possibly because each of the MSP methods has a different effect on trophic conditions [21,34]. As a result of mineral soil exposure in farrows formed by FP, the concentrations of nitrogen and cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in the rooting zone of seedlings are two- to threefold lower than in belts created by FM [46]. Supposedly, nutrient content of the soil at the bottom of furrows formed by AP (in which a small amount of humus is mixed with mineral soil) is intermediate between FP and FM. That is probably why seedlings in FP plots were shorter and thinner than those in FM plots, whereas those in AP plots were of intermediate size. However, the influence of MSP method on seedling growth was significant in first three years and disappeared in the fourth year of the experiment.

The fast growth in the first years after reforestation is important because of competition with other plant species [24]. Planted pine seedlings grew taller than weeds in the third year of the experiment, whereas seedlings from natural regeneration and direct seeding attained this growth a year later. Individual MSP methods more or less limit the growth of herbaceous vegetation in the clear-cut area [33,47]. In FM plots, vegetation left in undisturbed belts shades the seedlings and may compete with them for nutrients to some extent [36,38]. Regeneration of vegetation after site preparation with FP and AP should not occur before the end of second or even third growing season [33,48]. Differences in the rate of soil colonization by weeds, depending on MSP method, are maintained in successive years. In older plantations, after 6–7 years of cultivation, dry biomass of herbaceous vegetation in a clear-cut area prepared by FM can be 5- to 10-fold higher than in FP plots [46]. Weed growth in a forest plantation reduces not only tree growth but also survival rate [15]. For this reason, in young forest plantations in Poland weeds are routinely mown once, twice or even three times a year, if necessary. Consequently, the costs of vegetation control after site preparation by FM are higher than in AP and FP plots. Similarly, vegetation control in plots with planted seedlings, which grow faster than seedlings from natural regeneration and direct seeding, will be less expensive, as they need weeding for a shorter time.

Most of the earlier studies showed that bare mineral soil is an optimum seedbed for forest tree germination [31,49,50]. In such conditions, seeds have better contact with the soil surface and hence better moisture conditions (because of capillary water transport to the soil surface), compared to the humus or organic horizon [51,52]. As a result, seedling density in natural regeneration in a clear-cut area is highest after site preparation with FP, lower after the use of AP and lowest on the belts made by FM [38]. Our results confirmed this relationship only in the second year, in relation to seedlings self-sown in the second year in natural regeneration. The number of seeds that germinated in FP plots exceeded by more than threefold those in FM plots and by more than twofold those in AP plots. In the first year of the experiment, conditions for seed germination, creating different types of seedbeds, were similar in FP and AP plots and better than those created in FM plots. Apparently, our results were markedly shaped by moisture conditions. Spring in 2008 was characterised by high precipitation in April (60 mm) and May (94 mm) as well as favourable AI: 38.1 and 49.3 respectively during seedling emergence [53,54]. This ensured a high moisture content of the furrow bottom in AP plots (with a mixture of mineral soil and humus) and lower in FM plots (with a mixture of crushed herbaceous plants, forest litter, humus and mineral soil). In the second year of the experiment (2009), the AI value in April (4.7), as well as in July (13.7), August (17.5) and September (15.9), indicated drought. Consequently, seedling density was highest in FP plots, where seed germination and seedling development took place in furrows with exposed mineral soil.

Seedling density in natural regeneration in the first year of our experiment, in spite of favourable weather conditions (temperature and precipitation), was very low: 11,265 seedlings ha^{-1} in FP, 6077 seedlings ha^{-1} in FM and 19,417 seedlings ha^{-1} in AP. Low seedling density was due mostly to low seed production. Seedling density increased remarkably during the second year, to 75,593 seedlings ha^{-1} in FP, 22,826 seedlings ha^{-1} in FM and 46,097 seedlings ha^{-1} in AP. Such densities seem satisfactory from the point of view of forest management [2], although they are lower than those reported in other Polish studies. In earlier research conducted by our team [38], seedling density reached 188,000 seedlings ha^{-1} in soil prepared by FP, 121,000 seedlings ha^{-1} in AP and 36,000 seedlings ha^{-1} in FM. In fact, the density can be even higher, up to 360,000 seedlings ha^{-1} [55].

In addition to seedling density, an important parameter of natural forest regeneration is a more or less even distribution of seedlings in the reforested area. An analysis of evenness of seedling density confirmed the existence of differences in conditions for seed germination and seedling growth between the first and second years of the experiment, dependence of seedling density on MSP method and low seed production in the first year. As a result, in the first year, zero plots (with no seedlings) were numerous in all variants (40.0 per cent in FP, 26.7 per cent in AP and 42.2 per cent in FM), but their number markedly declined in the second growing season as a result of germination of additional seedlings. The most favourable evenness of seedling density and a lack of zero plots from the second year until the end of the experiments were recorded in FP plots, so this MSP method is the most favourable for natural regeneration. Apart of the main tested factors (MSP methods), the high rate of zero plots could be also associated with microhabitats that differ in a soil moisture content and/or the random nature of self-sown reforestation method.

Our results confirm that the major factors affecting natural regeneration of pine forest stands are seed yields and favourable weather conditions during seed germination and initial seedling growth [56,57].

Although seedling density was highest after direct seeding, seed losses were high in all MSP variants. Seedling density after the first year indirectly shows the magnitude of the losses. In FM belts, seedlings developed from only 35 per cent of sown seeds and from 46.5 per cent of FP furrows. Conditions for seed germination and seedling survival in the first year were the best in AP plots (53 per cent). Seed losses after direct seeding are often very high [53,58], which is a disadvantage of this reforestation method. Seed germination depends on many different factors: seed quality, temperature and moisture conditions [51]. It is also affected by adverse factors such as seed predation and mortality due to pathogens [59]. Although pine seeds germinate better in light [53], covering seeds protects them from water losses caused by evaporation [60] and consumption by animals [59]. In our experiment, temperature and moisture conditions were favourable for seed germination. AI in April and May reached 38.1 and 49.3, respectively. Pine seeds can germinate at 5 °C, but the optimum temperature range is 20–25 °C [61]. After sowing (15 April), daily mean temperature did not fall below 6 °C; the monthly mean temperature was 9 °C in April and 12.9 °C in May. Seed losses in our experiment could have resulted from insufficient exposure to light or poor seed quality. In Polish conditions, the germination of seeds collected in the stand without pre-selection is ca. 60% [62].

A high contribution of zero plots usually reflects a high microhabitat variation of a clear-cut area [63]. After direct seeding, the percentage contribution of zero plots was relatively low but depended on MSP method. It was the highest (8.9 per cent) in the FP variant. The surface of mineral soil usually creates good moisture conditions for germination [51]. Covering of seeds in this case could cause oxygen deficits in more humid microhabitats [60]. The highest seedling density and most favourable evenness of seedling density during 4 years of growth were observed after soil preparation with AP. Thus it can be assumed that this MSP method is the most suitable for direct seeding.

Planted pine seedlings were characterized by a higher survival rate than directly seeded and self-sown seedlings. After 4 years the difference reached nearly 25 percentage points. MSP method did not have any significant effect on seedling survival rate. Mäkitalo [9] reported similar results on dry pine-dominated sites. Reforestation by direct seeding and natural regeneration, at early stages of development, is linked with high numbers of seedlings but also their high mortality [43]. Seedling mortality is affected by their high density and the resultant competition among them, small size of the seedlings, competition with neighbouring vegetation, extreme climatic events, diseases and insect infestation and browsing [11,43,54,64]. In our experiment, pine survival was influenced by seedling size. Like Wennström et al. [65] and Collet and Moguedec [43], we found that larger seedlings had a higher survival rate. It correlated with both seedling height and thickness, although this relationship changed depending on seedling age and the parameter analysed. Survival rate was significantly correlated in the second year only with root collar diameter, in the third year by both parameters and in the fourth by seedling height only. Similarly, in natural regeneration, the seedlings that germinated in the first year were larger and more likely to survive that those germinating in the second year of the experiment. Survival rate after 2 years from germination was close to 80 per cent for older pine seedlings and nearly 45 per cent for younger ones.

Many studies show that seedling mortality can be due to excessive density [43,64], but during the 4 years of our experiment seedling density did not significantly affect survival rate in any year. This result could be explained by the relatively low density of seedlings. Herbivore damage to the seedlings was prevented by protection with sheep wool and fencing the plots, and hence seedling survival rate was not affected by herbivore damage in these experiments.

Our results confirmed that, for natural regeneration under unfavourable moisture conditions, more intensive soil scarification methods usually result in a higher initial number of seedlings and better subsequent growth and survival [31–34]. Better results from less intensive MSP methods (AP, FM) in the directly seeded and planted variants, as well as in natural regeneration in the first year of our experiment, were probably a consequence of favourable weather conditions, especially of sufficient precipitation [54] and a lack of competition with the herb layer of vegetation [49].

MSP can significantly enhance the risk of soil erosion [23,24]. The three mechanical site preparation methods used in our study are characterised by different levels of soil scarification, from very intensive performed by FM, through medium in case of AP, up to low soil disturbance made by FM. Thus, for each of listed above MSP methods, different levels of soil erosion risk can be expected: the highest in case of FP, lower in case of AP and minimum one for FM. However, the soil erosion risk is highly associated with uplands and mountains regions. In our case, the study area was flat and not enough large to observe any results of water and wind erosion respectively. Also the soil type was not enough pure and sandy to trigger the wind erosion processes. The areas that are at risk of erosion and invasive species should be treated with due caution in terms of MSP and reforestation methods. Based on our results we would propose to use FM in case of sites with soil erosion risk, while the FP should be applied in case of risk from invasive species. Under such conditions the planting of older seedlings is recommended instead of direct seedling and natural regeneration.

Finally, the costs of establishment and tending operations of each new regenerated stands can varied a lot, depending on the applied site preparation method and reforestation method. The relationship of costs in the case of MSP is 1 (180 $/ha):2.2:2.4 in case of FP, AP and FM respectively (source: the labour of work, in hours per 1 hectare, given in the Polish Standards for works to be done in the forest practice (2004) combined with the present gross incomes of enterprises, in PLN per 1 h and recalculated for USD per 1 h, that are employed in Narol Forest District). The much higher costs of the latter two methods are associated with required much stronger tractors (at least 100–120 KM while in case of FP just 80 KM), slower work output (e.g., in case of FM max. 0.8 ha per 8 h and up to 2 ha at the same time for AP and FP) and higher amortisation costs due to the presence of active parts that need to be replaced after 2–3 years of work. The costs of reforestation methods also differ a lot. Those costs can be completely avoided when the natural regeneration is applied. In turn, the costs of one year old seedlings from nursery (650 $/ha) and their planting (850 $/ha) are much more higher than costs of seeds (300 $/ha) and sowing (50 $/ha)—respectively 1:0.23. Although the cost of planting is greater than the other reforestation methods, planted seedlings exhibited greater survival and growth rates, suggesting more uniform and predictable results (as opposed to the patchy distribution of the natural regeneration method, for example). Thus planting is also more successful at competing with weeds, representing potential reduction in weed control costs in comparison to smaller seedlings from direct seeding and natural regeneration at the same age. We would also expect the lower costs of respacing in case of stands originated from planting. Finally, seedlings could be very successfully planted in the FM system, with very low soil treatment and likely low erosion risk and subsequent environmental costs. However, we should keep in mind the fact that methods exanimated in our study that are more environmental friendly (AP and FM) are at least double the price of a traditional one (FP).

5. Conclusions

Reforestation by planting of pine seedlings results in faster growth and higher survival rate compared with natural regeneration and direct seeding. Although MSP did not significantly affect the growth parameters and survival rate of planted pine during the first 4 years of growth, some clear trends in variation of the analysed variables are noticeable. As a result of soil preparation by FM, planted seedlings grow only slightly faster than in AP and FP plots, and their survival rate is slightly lower than in FP plots. Thus FM can be recommended for coniferous forest sites with poor potential (low soil fertility), where competition with herbaceous vegetation is low. The major advantage of this method is that it affects the soil environment to a much smaller extent than FP and AP, as the belt disturbed by FM accounts for only 27 per cent of the surface area.

Natural regeneration and direct seeding should be promoted as more environmentally friendly than planting, although they are associated with a high risk of failure. In the first year, in spite of favourable weather conditions, in natural regeneration plots a level of seed production from mature trees (seed yield) resulted in very low seedling density, so the percentage contribution of zero plots was high in all MSP variants. In the second year, when moisture conditions were less favourable but seed yield was high, seedling density was highest and most homogeneous after soil preparation with FP. We conclude that the use of FP for soil preparation in plots set aside for natural regeneration is associated with the lowest risk of failure.

In directly seeded plots, seedling density differed significantly depending on the MSP method. It was highest (no plots without seedlings) and most homogeneous after the use of AP. The other variables (growth parameters and survival rate) were similar for all MSP methods. Thus AP can be recommended for directly seeded plots in southern Poland, where precipitation is high.

In the directly seeded and self-sown variants, seedling density was lowest (many plots with no seedlings) and least homogeneous after soil preparation with FM. This indicates that crushing and mixing of herbaceous plants, forest litter, humus and mineral soil on the belts made by FM, irrespective of weather conditions, does not create favourable conditions in terms of soil water availability for seed germination and seedling growth. Soil preparation with FM in clear-cut areas for direct seeding and natural regeneration is associated with a high risk of failure.

This is the first research to be carried out on Scots pine forests in Poland, and as far as we know also in Europe, to compare at once three methods of forest reforestation and three methods of site preparation, used experimental design. The results from this paper therefore contribute to the existing silvicultural knowledge on Scots pine forest reforestation in Europe. Furthermore, results obtained will be of value to forestry practitioners in making decisions concerning the optimal choice of the site preparation method and reforestation method, whilst taking into consideration the local climatic conditions. The findings have also implications for improving the cost-effectiveness of Scots pine reforestation in Poland, as well as in other European countries with similar site and comparable climatic conditions.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/8/11/421/s1: Figure S1: Evenness of seedling density (%) for Scots pine after natural regeneration in relation to MSP methods; Figure S2: Evenness of seedling density (%) for Scots pine after direct seeding in relation to MSP methods; Table S1: Results of 2-way ANOVAs describing the statistical significance of the effect of reforestation and of MSP methods on height, root collar diameter and density of Scots pine seedlings after four growing seasons; Table S2: Regression equations describing the relationships between height increments and height and between root collar diameter increments and root collar diameter of Scots pine seedlings.

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