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Survival and growth of Scots pine (*Pinus sylvestris)* seedlings in north Sweden: effects of planting position and arginine phosphate addition

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ABSTRACT
Forest regeneration by tree planting on harvested sites in the boreal forests of northern Europe is frequently preceded by site preparation to increase survival and growth of the seedlings. We studied whether a small addition of arginine phosphate (AP treatment) at the time of planting would further enhance the seedlings' early performance. Following two growth seasons, we investigated survival and growth of Scots pine (*Pinus sylvestris)* seedlings on 11 locations between latitudes 61.1°N and 67.1°N in the boreal forest of northern Sweden. The planting positions of seedlings were on capped mounds and bare mineral soil following mechanical site preparation, and in non-prepared soil. We found that seedling survival following site preparation increased with AP treatment. On capped mounds, seedling survival was more variable and appeared more dependent on precipitation during the first month after planting than seedlings positioned in the mineral soil. The positive effect of AP treatment on seedling growth differed between sites and was more pronounced on sites with longer growing seasons. AP treatment had no significant effect on survival of seedlings planted in non-prepared soil, while the positive effect on growth was more pronounced at sites with higher fertility using this planting position.

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KEYWORDS
Pinus sylvestris; forest regeneration; seedling survival; seedling growth; planting positions; arginine phosphate

Introduction

Forestry practice in the Nordic countries involves the planting of tree seedlings on harvested forest sites. The environment on such clear-cuts is challenging for the seedlings. To improve their survival and growth, mechanical site preparation is used. Mounding and disc trenching are the two most common mechanical site preparation methods used in Sweden. Elevated planting positions are produced as isolated mounds in rows when mounding by excavator is carried out, while elongated continuous berms are produced by disc trenching. When successfully completed, the resulting elevated planting areas following both mounding and disc trenching consist of an inverted humus layer positioned on underlying intact humus and topped by mineral soil. The terminology used regarding mineral mounds on inverted organic matter may vary depending on method, country where the method is practiced and author (Sutton 1993). Here, we use the term "capped mound" for both isolated mounds and continuous berms, where "capped" implies a mineral soil cover over a mound of organic matter (Sutton 1993) and thus accurately describes the resulting elevated planting positions produced by both disc trenching and mounding. Capped mounds are the recommended planting positions in Swedish forestry (Skogsstyrelsen 2020), mainly because nutrients released during decomposition of the embedded organic material are beneficial to seedling growth. Furthermore, the raised position is warmer and less exposed to frost damage and flooding than a lower one (Örlander et al. 1990; Langvall et al. 2001; Burton et al. 2000). On the other hand, capped mounds can suffer from low soil moisture conditions because the organic layer within them reduces capillary water flow from below (Örlander et al. 1990, 1998; Burton et al. 2000; de Chantal et al. 2003). Also, variation in soil type, the occurrence of large rocks, stumps and logging residues on the clear-cut site can cause a large variation in the quality of the capped mounds, even within a single site (Sutton 1993; Larsson 2011; Söderbäck 2012; Sundström 2021). Mechanical site preparation is generally carried out the year before planting to allow the capped mounds to be compacted by snow. Nevertheless, if there are many branches, rocks or dense ground vegetation embedded within the capped mound, the contact with underlying soil and access to capillary water can yet be compromised (Örlander et al. 1990; Grossnickle 2005). Thus, an individual quality assessment is made for every capped mound at the time of planting. It is generally recommended to plant deep, preferably through the organic layer (Örlander et al. 1990). However, it is not a trivial matter to judge whether a capped mound provides a suitable planting position or not. It is not always possible to assess the depth of the mineral cover of a capped mound externally and it may not always be practically possible to position

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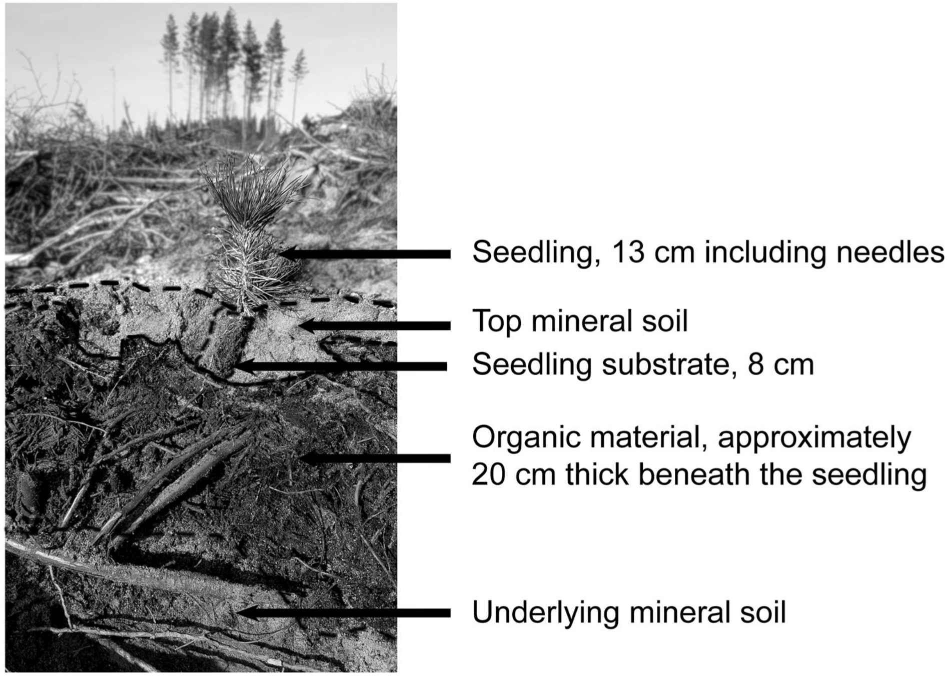

Figure 1. A cross section of a capped mound that would be classified as optimal when looking from above ground. The cross section reveals that the planting position is not optimal for the seedling to reach capillary water. Dashed lines mark the top surface of the capped mound, top and bottom of the organic layer and the outline of the seedling substrate. The seedling substrate barely reaches through the top mineral layer and is far above the underlying mineral soil. This specific capped mound was not from a trial site included in this study, but the aim of this figure is to provide an example of how the interior of a capped mound might look.

the seedling correctly i.e. through the organic layer so that the roots reach the mineral soil to access capillary water (Figure 1). Sometimes the depth of planting can be limited by physical obstacles beneath the surface, such as a twigs or rocks, or by seedling size. The most common seedling stock types planted in northern Sweden are grown in containers with a cell size of 30 or 50 cm^3. Pine seedlings grown in these containers are often no more than 10 cm tall when planted. If planted too deep, these seedlings would have a very low proportion of the shoot above ground which could, potentially, negatively affect growth (Johansson et al. 2015).

Climate change scenarios predict both increased temperature and precipitation in Sweden (Strandberg et al. 2015). Despite the increase in precipitation, a reduction in water availability is expected in many areas of Sweden during the summer due to increased evaporation (Eklund et al. 2015). With increasing evaporation, there will be an increasing risk of seedling desiccation and water stress-induced mortality. Seedlings planted in capped mounds are particularly susceptible in such scenarios, since low soil moisture conditions decreases water uptake ability of seedlings more in capped mounds than in pure mineral positions (Örlander 1986). At present, the recommendation when planting during dry weather conditions in south Sweden is that seedlings should be planted at a relatively high position in the mineral soil exposed by the soil scarification, while in north Sweden the recommendation is to plant in the capped mound regardless of weather conditions (Skogsstyrelsen 2020). However, planting in mineral soil can potentially lead to reduced growth due to the low nutrient availability. Nitrogen availability is often limited in boreal forests, where most of the plant-available nitrogen is found in the humus layer (Tamm 1991; Grossnickle 2000; Bhatti et al. 2005).

The addition of a long-term release nitrogen source at the time of planting could potentially compensate for the lower nitrogen availability in the mineral soil (Brand 1991; Thiffault & Jobidon 2006). Fertilizers based on inorganic nitrogen, such as ammonium and nitrate, are the most common commercially available ones, but nitrogen is naturally mainly available to plants in organic form i.e. amino acids in boreal forests (Inselsbacher & Näsholm 2012). The amino acid arginine is synthesized by coniferous trees and also many vascular plants to enable internal storage of nitrogen in foliage or other plant parts (Nordin et al. 2001). Arginine has the highest nitrogen content of the amino acids (Cánovas et al. 2007). In *Pinus sylvestris* L., arginine is the dominant constituent of the amino acid nitrogen pool in needles, twigs and bark, and a major constituent, along with glutamine, in the wood (Nordin et al. 2001). When nitrogen uptake exceeds levels the trees can utilize for growth, the arginine levels increase in needles and wood (Edfast et al. 1996; Nordin et al. 2001). This storage is then utilized by plant metabolic processes to provide nitrogen required for early season growth (Canton et al. 2005). Also, in forest soils, amino acids act as an organic nitrogen source accessed by plant roots (Öhlund and Näsholm 2001; Gruffman et al. 2013). In soil, arginine is a strong cation and has a very high binding capacity to soil particles (Inselsbacher et al. 2011). Consequently, arginine nitrogen does not leach from forest soils even when applied in relatively high doses (e.g.

Hedwall et al. 2018). Hence, an environmentally friendly and commercially available fertilizer based on arginine has been developed: arGrow® (Arevo AB, Umeå, Sweden). In arGrow®, the arginine is crystallized with phosphate and granulated to form a slow-release fertilizer. So far, most studies of fertilization with arginine have been in tree seedling nurseries and have shown that conifer seedlings treated with arginine develop a higher mean dry weight, a higher root-to-shoot ratio as well as a larger proportion of root tips colonized by mycorrhiza, compared to seedlings treated with inorganic nitrogen fertilizers (Öhlund and Näsholm 2002; Gruffman et al. 2012).

The aim of this study was to evaluate the effects of adding arginine phosphate (arGrow®) on the field performance of *P. sylvestris* seedlings in different planting positions. We used a large field trial, across 11 clear-cut forest sites between latitudes of 61.1°N and 67.1°N in northern Sweden. The experiment was carried out on multiple commercial forestry sites which offered a wide range of environmental conditions to mimic "real life" conditions. This approach exploits the different combinations of environmental variables present at each site. Some variables are related to natural variation, such as geographical location, soil type and climate, while others are related to silviculture practices, such as site preparation method and site preparation performance along with seedling features such as stock type, seed source, seedling size and nursery regime. Many of these variables and their combinations can potentially affect seedling performance in the field (Burdett 1990; Margolis & Brand 1990; Grossnickle 2012). However, this broad span of site conditions is also the strength of this study since the main goal was to achieve results that were practically applicable to a great range of commercial site conditions rather than to controlled experimental conditions.

The effect of arginine phosphate treatment (AP treatment) was evaluated for seedlings planted in capped mounds, the adjacent exposed mineral soil and in non-prepared soil. Many previous studies have pointed out that seedling performance in non-prepared soil is normally significantly lower than that in scarified soil, but the practice may still be interesting on sites with particularly sensitive ground vegetation, such as reindeer lichens. Also, seedling performance data were correlated with weather (precipitation), climate (length of growing season) and site fertility conditions (site index). The effects of these variables on seedling growth and survival in different planting positions and treatment combinations were evaluated.

The main objectives of this study were (i) to evaluate the effect of arginine phosphate (AP) treatment at the time of planting on seedling performance in different planting positions over multiple sites in northern Sweden; (ii) to evaluate the effect of climate variation across sites on seedling performance in the different planting position and treatment combinations and (iii) to evaluate the potential of arginine phosphate as a tool to compensate for the lower nutrient availability in mineral soil as compared to capped mounds, where nutrients are available from decomposing organic material.

To address these objectives, we formulated the following hypotheses:

1. AP treatment at the time of planting will positively affect survival. We expected that the positive effect of arginine phosphate on root growth and mycorrhizal colonization (Öhlund and Näsholm 2002; Gruffman et al. 2012) would enhance survival since extension of the root system can increase the water uptake capacity of seedlings (Bréda et al. 2006; Brunner et al. 2015).
2. Low precipitation during the seedling establishment period will affect survival negatively, particularly for seedlings positioned on capped mounds. We expected that seedlings on capped mounds would exhibit a higher dependence on precipitation in comparison to seedlings in bare mineral soil. This would be due the restricted access to capillary water from below compared to the more direct access to capillary water in mineral soil (Örlander et al. 1990, 1998; Burton et al. 2000; de Chantal et al. 2003).
3. AP treatment will enhance seedling growth. We expected that the positive effect of arginine phosphate on root growth and mycorrhizal colonization (Öhlund and Näsholm 2002; Gruffman et al. 2012) as well as the direct access to nitrogen would enhance shoot growth.
4. AP treatment of seedlings planted in bare mineral soil will exhibit similar growth in this position to that in capped mounds. We expect that the direct access to nitrogen through the long-term release nitrogen source (Brand 1991; Thiffault and Jobidon 2006) will facilitate increased growth in the otherwise nitrogen-limited environment (Tamm 1991; Grossnickle 2000; Bhatti et al. 2005).
5. Seedlings will perform better after mechanical site preparation than in non-prepared soil. Site preparation is known to enhance seedling performance by improving micro-site conditions to favor establishment of the newly planted seedlings, such as increased temperature, decreased competition from ground vegetation and decreased damage from pine weevil (Örlander et al. 1990).

Material and methods

Field experiment design

A field experiment to evaluate the effect of arginine phosphate (AP) treatment on Scots pine (*Pinus sylvestris* L.) seedlings in different planting positions was set up during spring and early summer in 2018. The seedlings were split into two treatment groups: (a) treated with AP: one dose of granular arginine phosphate (arGrow® Granulat, Arevo AB, Umeå, Sweden) was added to the bottom of the planting hole together with each seedling at the time of planting; (b) untreated: no nutrients added. One dose of arGrow® Granulat contains 40 mg N and 22 mg P, the active substance being L-arginine phosphate ($C_6H_{17}N_4O_6P$). The seedlings in each treatment group were planted in three different positions: (i) capped mound i.e. turned-over humus tilt with a mineral soil cover on top of intact humus (Figure 1), (ii) mineral soil i.e. bare mineral soil adjacent to the capped mound where the topsoil had been removed, and (iii) non-prepared soil i.e. undisturbed intact humus where no topsoil or vegetation had been removed. Planting positions

Table 1. Sites A–K are listed from south to north with latitude (Lat.), longitude (Long.), and altitude (Alt., meters above sea level). Volume (vol.) refers to the cell size of the growing containers, which is the volume the seedlings' roots were restrained in at the time of planting.

Site	Lat. (° N)	Long. (° E)	Alt. (m.a.s.l.)	Vol. (cm^3)	Method	Precipitation first 30 days (mm)	Growing season (days)	SI	No. leader shoot length measured
A	61.06	16.16	360	50	DT	35	165	T24	109
B1	62.07	15.09	260	30	DT	36	155	T20	66
B2	62.07	15.09	260	50	DT	36	155	T20	144
C	63.95	18.45	260	50	M	24	145	T22	62
D	63.95	18.44	280	50	DT	24	145	T20	42
E	64.18	19.91	180	50	DT	44	145	T22	14
F	64.26	19.61	180	50	DT	45	145	T21	81
G	64.31	19.64	300	50	DT	44	145	T22	68
H	65.62	20.63	180	30	M	43	140	T19	64
I	66.38	21.82	200	30	DT	43	135	T19	88
J	66.64	20.30	260	50	M	48	130	T16	107
K	67.09	22.30	200	30	DT	13	130	T18	288

Method refers to which mechanical site preparation method was used for each site, disc trenching (DT) or mounding (M). Environment parameters: Total precipitation during the first 30 days after planting in 2018 (SMHI, 2019), length of growing season in days (SMHI 2020) and site index (SI). The "T" in site index indicates pine sites in Swedish site index classification (Hägglund & Lundmark 1987). No. leader shoot length measured = the total number of seedlings measured for each site. Site B include seedlings grown in both containers with cell volume 30 cm^3 (B1) and 50 cm^3 (B2) and is therefore divided into two subsets.

(i) and (ii) were both created during mechanical site preparation carried out in 2017. The mechanical site preparation methods were disc trenching at eight sites and mounding at three sites (Table 1). Planting was carried out by experienced planters. Planting in capped mounds was only carried out where the capped mounds had appropriate mineral soil cover, so when the planting position was classified as "good".

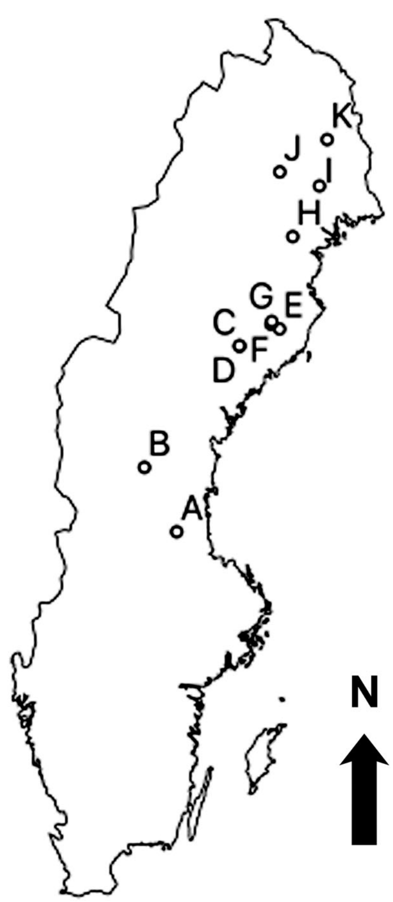

Figure 2. Distribution of sites within the boreal forest area of northern Sweden between latitudes 61.1°N and 67.1 °N.

The experimental plots were spread over multiple sites in Sweden between latitudes 61.1°N and 67.1°N (Figure 2). The soil moisture class was dry on all sites except site C which was mesic. Soil types varied between silty, sandy and coarse till, where larger particle sizes (i.e. gravel and bigger rocks) were present in the soil at all sites. Each site represented a combination of many different environmental variables resulting from a combination of natural variation and silvicultural practices (Table 1). In this study, precipitation during the first 30 days after planting, length of growing season and site index were the variables that showed the most significant correlation to survival and/or growth performance and were therefore the variables chosen to represent site variation (Table 1). Site index (SI) represents the productivity of the sites and is the estimated height of dominant trees at 100 years based on the productivity of the former stand.

At each site, 2–4 rows of seedlings with each planting position and treatment combination were planted on areas with relatively homogeneous terrain. For each track made by either mounding or disc trenching, all three planting positions were used i.e. capped mound and mineral soil positions in the track and non-prepared soil between tracks. The rows were arranged adjacent to each other, so three rows with AP-treated seedlings (one for each planting position), and the next three adjacent rows with untreated seedlings for each planting position, repeated 2–4 times. Due to lack of good planting positions in capped mounds on many sites, varying numbers of seedlings were planted in each position and treatment combination for each site. For details of numbers of planted seedlings, please refer to the supplementary material (Table 7).

Seedling material

Seedlings of *P. sylvestris* from different nurseries were used on different sites/groups of sites depending on the provenance and site owner. Each site was planted with seedlings grown in containers with either 30 cm^3 or 50 cm^3 cells, except for one site (B) which was planted with both sizes (Table 1). For further seedling material details, please refer to the supplementary material (Table 6).

Climate variables

Precipitation data were retrieved from the nearest available Swedish Meteorological and Hydrological Institute (SMHI) weather station (mean distance 18.5 km, maximum distance 30 km) database for each site (SMHI 2019). The length of growing season is the normal value based on 1961–1990 data, where the start of growing season is defined by the first day of the year when the diurnal mean temperature has been above 5°C for four consecutive days, and the end is the last day of the last four days period when the diurnal mean temperature has been below 5°C (SMHI 2020). Precise data for the length of growing season for the new normal period based on data from 1991 to 2020 are not yet available. However, the length of growing season has generally increased all over northern Sweden since 1990 (SMHI 2020) and so we expect that to be true for all the sites included in this study. Therefore we assume that the internal relations between the sites regarding length of growing season have not changed dramatically and that we thereby can relate site-dependent differences in growth and survival to the currently available data.

Inventory methods

A field inventory was carried out at the end of the second growing season during August and September 2019. All seedlings with any green needles were classified as living, seedlings with no green needles, and missing seedlings were classified as dead. Cause of death was not determined since this was not an aim of the study, but the majority of the dead seedlings were ones that were missing. For seedling growth, we used the current year (2019) leader shoot length to represent performance in the field since planting. The leader shoot length was measured from the top branches to the top of the terminal bud. The leader shoot of every second live and undamaged seedling was measured, randomly starting at the first or second seedling in each row. The majority of damaged seedlings lacked dominant leader shoot, often resulting in "brushy" seedlings with multiple leader shoots. The cause was not always possible to determine, but in many cases the leader shoot was removed by browsing. In northern Sweden, browsing by moose in late winter is a common cause of damage to young pine trees (Söderbäck 2012; Bergqvist et al. 2014). Leader shoot damage can also be caused by harsh winter conditions, such as temperature drops during low snow-cover, and have also been found to increase with low precipitation the first weeks after planting (Luoranen et al. 2018). At sites where many seedlings were damaged or dead in any of the planting positions and treatment combinations, all the remaining undamaged seedlings were measured from that combination.

There was a large variation in the number of measurement replicates taken from the 2019 inventory, with a total of 1207 seedlings being measured (Table 1). Varying numbers of seedlings planted at all sites and in all planting positions, variations in survival rates and numbers of damaged seedlings together with part-harvests of entire blocks for other purposes than this study in 2018 at several sites contributed to this.

Data selection and structure

Site C is not included in the analyses of seedlings planted in non-prepared soil since no seedlings were planted in this position at this site. Site A is excluded from the growth variable statistics for non-prepared soil because there were very few measurements due to low survival numbers. Site E was not included in the growth measurement analysis since a high number of damaged seedlings resulted in a very low number of available seedlings to measure in all planting positions. At site B, which was planted with two different seedling sizes, survival analyses only include seedlings of the larger size due to missing survival data for the smaller sized seedlings. However, measurement data include both seedling sizes separated in two datasets for this site.

Seedlings planted in mineral soil and capped mounds were analyzed in the same dataset since the main interest of this study was to compare the performance of seedlings in these two planting positions. Performance of seedlings in non-prepared soil is naturally affected by competition from other vegetation to a greater degree than the seedlings in mechanically prepared soil. Seedlings planted in non-prepared soil were, therefore, analyzed separately to avoid interference with the very different growing environment in the comparison to the mechanically-prepared planting positions.

Analysis methods

We tested the effects of the factors planting position and arginine phosphate treatment as well as the interaction between these factors. Therefore, we chose to use factorial ANOVA since this method can be used to find whether there is any significant effect of each factor and whether there is interaction between them (McDonald 2014; Mangiafico 2015). To account for any difference in effects of planting position and arginine phosphate treatment between sites, we used "site" as a third factor. R-studio (version 1.3.1093) software was used for all statistical analyses (R Core Team 2019). Analysis of variance (ANOVA) was performed for survival and growth using the R car-package (Fox and Weisberg 2019). Generalized linear models (GLM) were used to analyze survival, using survival log-odds (ratio of the probability of survival to probability of death) as the response variable. Growth was analyzed with linear models using leader shoot length as the response variable. To detect whether there were any interactions between the main factors, model III ANOVA was used as this model is recommended for unbalanced designs (Logan 2011; Walker 2018). In cases where no interaction between factors was detected, a follow-up model II ANOVA was carried out since model II is considered more powerful when no interaction is found (Langsrud 2003). The confidence level used in all analyses was 0.95. In the case of interaction between site and any of the other factors, the effect of site was further explored by fitting models separately for each of the levels in the other factors (Logan 2011). Each site represents many different environmental variables, such as amount of precipitation, temperature sum, length of growing season, site index etc. Each of these variables were tested to find which one represented the site effect best.

Generalized linear models were used to illustrate the relationship between (i) survival and precipitation during the first 30 days after planting in capped mounds and mineral soil and (ii) survival in non-prepared soil and length of growing season. Linear models were used to analyze site variation in growth in relation to (i) length of growing season for seedlings planted in capped mounds and mineral soil and (ii) site index for seedlings planted in non-prepared soil.

Results

Seedling survival in capped mounds and mineral soil

Treatment with arginine phosphate (AP treatment) at the time of planting had a significant positive effect on seedling survival after two seasons in the field, that is, the positive effect of AP treatment on seedling survival occurred independently of site and planting position (Figure 3(A), Table 2). The positive effect of AP treatment on survival appeared to be larger when the seedlings were planted on the capped mounds than when planted in the mineral soil (Figure 3(A)). The effect of planting position on seedling survival depended on the site as there was a significant interaction between the two variables (Figure 3(A), Table 2). Survival, averaged over all

Table 2. Results from ANOVA analysis of the effects of site, arginine phosphate (AP) treatment, planting position following mechanical soil preparation and the significant interactions between these variables on seedling survival following two growing seasons in the field.

	LR Chisq	Df	Pr (>Chisq)
Site	114.17	10	**<0.001**
Treatment	5.01	1	**0.03**
Position	0.00	1	0.99
Site x Position	173.72	10	**<0.001**

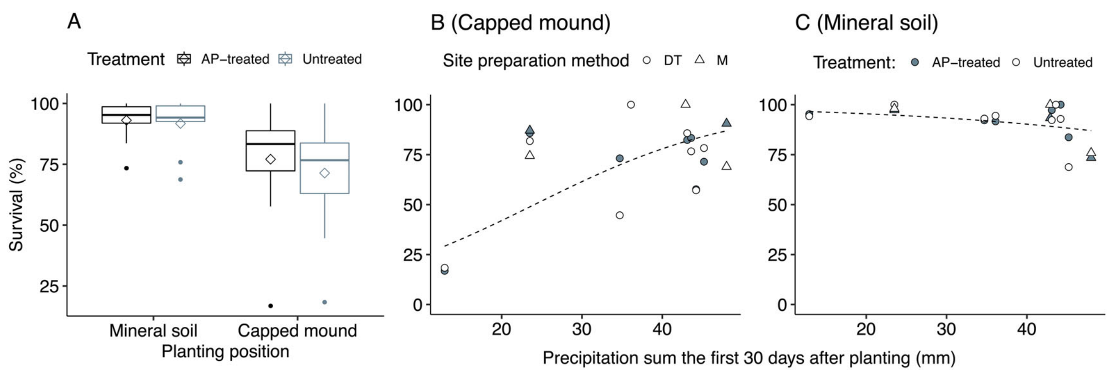

Figure 3. (A) Box and whisker plots of the observed proportional survival range of seedlings planted in mineral soil and on capped mounds with (black) or without (gray) arginine phosphate (AP) treatment across eleven study sites along a north-south gradient in northern Sweden. The diamonds indicate mean values for each position and treatment. The horizontal line in the boxes indicates the median survival value, that is, the value that is in the middle of all observed values. The boxes indicate the range between the lower quartile and upper quartile of the observed values i.e. ~50% of the values for each group are distributed within the boxes. The whiskers (vertical lines) above/below boxes indicate the maximum and minimum values that are not extreme values. Unconnected points outside the boxes represent extreme values that are outside 1.5 times the interquartile range above the upper quartile and below the lower quartile i.e. potential outliers. (B) Proportional survival in relation to precipitation during the first 30 days after planting in capped mounds and (C) mineral soil. Each site is represented by two survival values, one for AP-treated seedlings and one for untreated seedlings. Triangles mark out sites prepared by mounding and circles mark out disc trenched sites. The dashed lines represent the predicted curves from logistic regression models for each of the two planting positions. The gray areas represent the 95% confidence interval for each model.

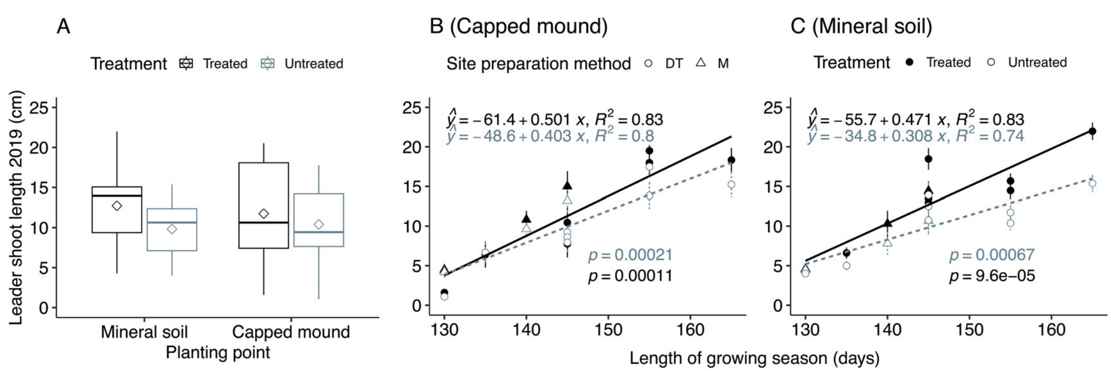

Figure 4. (A) Box and whisker plots of the range of observed mean leader shoot length values of seedlings planted in mineral soil and on capped mounds with (black) or without (gray) arginine phosphate (AP) treatment across eleven study sites along a north-south gradient in northern Sweden. The diamonds indicate mean values for each position and treatment. The horizontal line in the boxes indicates the median of the leader shoot length mean values i.e. the value that is in the middle of all observed values. The boxes indicate the range between the lower quartile and upper quartile of the observed values i.e. ~50% of the values for each group are distributed within the boxes. The whiskers (vertical lines) above/below boxes indicate the maximum and minimum values. (B) Linear relationships between leader shoot length and length of growing season for AP-treated (black text and line) and untreated seedlings (gray text and dashed line) in capped mounds and (C) mineral soil. Points indicate mean values for AP-treated (filled) and untreated (unfilled) seedlings for each site and bars indicate standard error. Triangles mark out sites prepared by mounding and circles mark out disc trenched sites.

Table 3. Results from ANOVA analysis of the effects of site, arginine phosphate (AP) treatment, planting position following mechanical soil preparation and the significant interactions between these variables on the leader shoot length of seedlings following two growing seasons in the field.

	Sum Sq	Df	F value	Pr (>*F*)
(Intercept)	71407	1	3843.73	<0.001
Site	20409	10	109.86	<0.001
Treatment	647	1	34.83	<0.001
Position	72	1	3.90	0.049
Site x Treatment	499	10	2.69	0.003
Site x Position	2114	10	11.38	<0.001
Treatment x Position	84	1	4.51	0.034
Site x Treatment x Position	84	10	0.45	0.919
Residuals	14955	805		

sites, was 71% for untreated and 77% for AP-treated seedlings in capped mounds and 92% for untreated and 93% for AP-treated seedlings in mineral soil. There was also less variation in survival between sites for seedlings in the mineral soil compared to seedlings on capped mounds (Figure 3(A)). Further analysis of the significant interaction between planting position and site revealed that the probability of survival for seedlings positioned on capped mounds increased significantly (p-value = <0.001) with the amount of precipitation at the different sites during the first 30 days following planting (Figure 3(C)), while this relationship was weaker but significantly negative (p-value = 0.01) for the seedlings positioned in mineral soil (Figure 3(B)). The models explained 52% of the variation in survival of seedlings planted in capped mounds and 25% of the variation in of seedlings planted in mineral soil.

Seedling growth in capped mounds and mineral soil

The length of leader shoot varied significantly between sites as well as positions and treatments, with significant pair-wise interaction between the three factors (Figure 4(A), Table 3). Further investigation of the site effect revealed that the length of growing season explained the main part of the site difference (Figure 4(B, C)). The positive effect of AP treatment increased with the length of the growing season, particularly for seedlings planted in the mineral soil (Figure 4(B, C)).

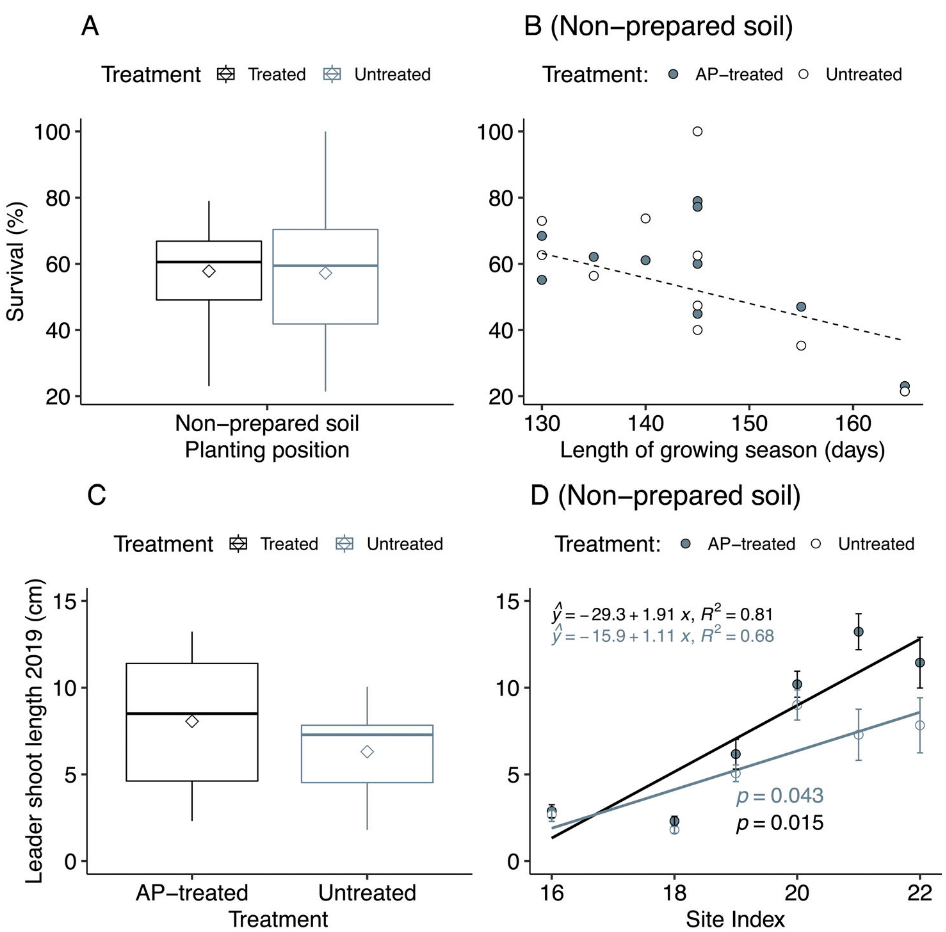

Figure 5. (A) Mean survival of seedlings planted in non-prepared soil with (black) or without (gray) arginine phosphate (AP) treatment across eleven study sites along a north-south gradient in northern Sweden. The diamonds indicate mean survival values for each position and treatment in (A) and mean leader shoot length mean values in (C). The horizontal lines in the boxes indicate the median survival value in (A) and mean leader shoot length mean values in (C) i.e. the value that is in the middle of the observed values for each treatment. The boxes indicate the range between the lower quartile and upper quartile of the observed values i.e. ∼50% of the values for each group are distributed within the boxes. The whiskers (vertical lines) above/below boxes indicate the maximum and minimum values. (B) Probability of survival in relation to length of growing season in non-prepared soil. Each site is represented by two survival values, one for AP-treated seedlings and one for untreated seedlings. The dashed lines represent the predicted curves from the logistic regression model. The gray areas represent the 95% confidence interval. (C) Mean leader shoot length of seedlings planted in non-prepared soil with (black) or without (gray) AP treatment. (D) Linear relationships between leader shoot length and site index for AP-treated and untreated seedlings in non-prepared soil. Points indicate mean values for sites with same site index and bars indicate standard error.

Table 4. Results from ANOVA analysis of the effects of site and arginine phosphate (AP) treatment on survival in non-prepared soil following two growing seasons in the field.

	LR Chisq	Df	Pr (>Chisq)
Treatment	0.03	1	0.87
Site	21.80	9	**0.01**

Table 5. Results from ANOVA analysis of the effects of site, arginine phosphate (AP) treatment and the significant interactions between these variables on leader shoot length in non-prepared soil following two growing seasons in the field.

	Sum Sq	Df	*F* value	Pr (>*F*)
(Intercept)	7372.7	1	1006.58	<0.001
Site	2781.2	8	47.46	<0.001
Treatment	109.6	1	14.96	<0.001
Site x Treatment	148.1	8	2.53	0.01
Residuals	1809.2	247		

Seedling survival and growth in non-prepared soil

In a separate analysis, we investigated the effects of AP treatment on survival and growth of seedlings planted in non-prepared soil. We found that seedling survival was, on average across all sites, 58% in non-prepared soil (Figures 3 and 5(A)). AP treatment had no significant effect on seedling survival (Figure 5(A), Table 4). Instead, we found a significantly negative influence (p-value = 0.01) on seedling survival of the length of growing season i.e. the shorter the growing season, the higher the seedling survival (Figure 5(B)).

There was a positive effect of AP treatment on seedling growth in non-prepared soil, but with a significant interaction between AP treatment and site (Figure 5(C), Table 5). The site index was the most important site variable, affecting growth in non-prepared soil, with the effect of AP treatment being more pronounced at sites with a higher site index (Figure 5(D)).

Discussion

Methods to improve the field performance of planting further are always being looked for as soil scarification and planting are the most expensive forestry investments made by a forest owner. In this study, we have demonstrated that treating pine seedlings with arginine phosphate (AP) at the time of planting can improve both seedling survival and growth. The effect of AP treatment on growth appears to increase with length of growing season for seedlings planted in capped mounds and mineral soil, and with increased site index for seedlings planted in non-prepared soil. We also demonstrated that survival is more variable between sites for seedlings planted in capped mounds than in mineral soil, and that any growth benefits of planting in capped mounds depends strongly on local site conditions. In addition, our results indicated that the mortality of seedlings planted in non-prepared soil increases with a longer growing season.

Supporting our first hypothesis, AP treatment had a positive effect on seedling survival in capped mounds and mineral soil across our 11 study sites along a north-to-south gradient over six latitudes in north Sweden. This positive effect of AP treatment on survival contrasts with findings by other studies of negative effects of nutrient addition when planting conifers, where inorganic N-P-K fertilizers were used (Simpson and Vyse 1995; Rose and Ketchum 2003; Thiffault and Jobidon 2006). The contrasting results may be related to the type of fertilizer used as well as to the dosages, which in the cited studies were 40–175 times higher than in our study. A high fertilizer salt concentration can harm root development which, in turn, negatively affects water uptake (Jacobs et al. 2004). The improvement of survival given by AP treatment could potentially be related to a positive effect of arginine phosphate on root growth and mycorrhiza colonization (Öhlund and Näsholm 2002; Gruffman et al. 2012). Both increased root growth and increased mycorrhiza colonization have been shown to increase the water uptake capacity for seedlings by extension of the absorbing surface of the root system (Bréda et al. 2006; Brunner et al. 2015).

Increased precipitation when the seedlings were establishing had a positive effect on the survival of seedlings positioned on capped mounds, which in part corroborated our second hypothesis. At sites with low precipitation, the differences in survival between seedlings planted in mineral soil and seedlings planted on capped mounds appeared to be larger than at sites with more abundant precipitation i.e. there was an indication that seedlings planted in mineral soil were more resistant to dry weather following planting than seedlings planted on capped mounds (Figure 3(B)). The effect of increased precipitation was negative for seedlings in mineral soil and hence this hypothesis was not corroborated for this planting position. The opposite trends of the curves suggest that seedlings planted in mineral soil are less sensitive to extreme drought, while seedlings planted on capped mounds seem less sensitive to high rates of precipitation. However, survival rates on sites with high precipitation are not exclusively higher for seedlings planted capped mounds. The relationship between reducing survival and increasing precipitation in mineral soil may be due to other unrelated effects, such as frost damage. The largest difference in seedling survival between planting positions was found at sites with lower precipitation during the establishment period. This finding emphasizes the difference between the two planting positions in respect of the risk to planted seedlings when exposed to drought. This variation in drought sensitivity depending on planting position might be one of the reasons why a large variation in survival between sites has been seen in other studies of forest regeneration in the Nordic countries (Hjelm et al. 2019; Sikstrom et al. 2020). The mortality of *P. sylvestris* seedlings has also been found to be strongly related to the number of dry days during the month the seedlings were planted (Sukhbaatar et al. 2020) and seedling mortality is associated with drought stress, even on sites where soil moisture is only low on rare occasions (Burton et al. 2000). The positive relationship between survival and precipitation during the first month explained approximately 50% of the variation in survival for seedlings planted in capped mounds in our model. This reflects that even if precipitation is important, it is not the only variable that affects survival. As Sikstrom et al. (2020) also emphasized, there are multiple causes behind this variation, such as other climatic factors, the mechanical site preparation that has a strong influence on the quality of the available planting area, plant material, handling of the seedlings and how well the seedlings were planted. In this trial, the planting was carried out by experienced planters and only planting positions regarded as good quality were used. The interior quality of the capped mounds was not specifically assessed since this would have been a destructive operation. However, the amount of logging residue could serve as an indicator for general quality of the capped mounds at a site. Smaller amounts of logging residues reduce the risk of a large amount of rough organic material becoming trapped within the capped mounds, thus giving better contact to the mineral soil below where the seedling can utilize capillary rising water. Site B was the only site where both seedling survival and growth were significantly better in capped mounds than in mineral soil. This site was not unique in relation to the combination of other site features, nor at either extreme of the climate variables listed, but it did have relatively smaller amounts of logging residues compared to sites with lower survival based on photographic evidence of the sites. Thereby, the quality of the capped mounds might have been higher at this particular site.

Our third hypothesis was that AP treatment would enhance seedling growth independent of seedling positioning, since increased N uptake is known to have a positive correlation with leader shoot growth in the following year (Grossnickle 2000; Nilsson 2020). We used the length of the leader shoot as an indicator for growth, but it should be noted that the AP treatment is primarily intended to improve the growth of roots and mycorrhiza (Gruffman et al. 2012) and, therefore, shoot length would be a secondary effect of the treatment. This hypothesis could not be confirmed as a general statement since the positive effect of AP treatment on leader shoot growth depended both on site conditions and planting position. However, our results indicated that the positive effect of AP treatment increased with a longer growing season for seedlings planted in capped mounds and in mineral soil. A stronger response to AP treatment was exhibited in mineral soil than in capped mounds. For seedlings planted in non-prepared soil, the site index rather than length of growing season explained the variation in seedling growth, and the growth promoting effect of AP treatment was more pronounced at more fertile sites with higher site indices. This might, as with the positive effect of AP treatment on survival in capped mounds and mineral soil, be related to better root growth and increased mycorrhiza colonization (Öhlund and Näsholm 2002; Gruffman et al. 2012) which would give seedlings planted in non-prepared soil an advantage over competing vegetation, thereby giving these seedlings a chance to benefit from the more fertile site.

We also hypothesized that AP treatment would compensate for the lower nutrient availability in bare mineral soil compared to capped mounds. This hypothesis could not be confirmed because our results indicated that the difference in performance between the two planting positions was highly dependent on site variables, in particular the effect of precipitation during the establishment period on survival, and the length of growing season on growth. Additionally, at most sites, both AP-treated and untreated seedlings planted in mineral soil grew equally well as, or even better than, seedlings planted in tilts. Only at one site (B) did seedlings grow significantly better when positioned in capped mounds. The expectations were that seedlings planted in capped mounds in general would grow better than in mineral soil and that the AP treatment would be needed for the seedlings in mineral soil to grow equally well. One reason behind the somewhat unexpected outcome could be that the summer of 2018 was exceptionally dry, and drought affects both survival and growth negatively (Burdett 1990; Örlander et al. 1990; Bréda et al. 2006; Luoranen et al. 2018). The lack of general superior growth in capped mounds is, however, not unique to our study. In a study by Hjelm et al. (2019), no significant difference in tree volumes was found after 30 years between trees planted in the mineral soil close to the berm after disc trenching and trees planted in capped mounds after mounding.

Our fifth and final hypothesis was corroborated, as survival and growth were both lower in non-prepared soil than in the planting positions resulting from mechanical site preparation. Survival was, on average, only 58% compared to the average survival observed in the mechanically prepared planting positions of 71 and 77% (untreated and AP-treated respectively) in capped mounds and 92 and 93% (untreated and AP-treated respectively) in mineral soil. In contrast to seedlings planted in the mechanically-prepared planting positions, AP treatment had no significant effect on survival in non-prepared soil. Furthermore, survival decreased with length of growing season in non-prepared soil. The negative correlation between survival and length of growing season in non-prepared soil could be seen as an indicator of increased competition from vegetation over the longer the growing season, and might also relate to lower pressure from pine weevil (*Hylobius abietis* L.) at more northly sites and further from the coast i.e. sites with shorter growing seasons (Björklund et al. 2014; Johansson et al. 2015). Both these factors are known to have a negative impact on seedling field performance (Örlander et al. 1990; Nordlander et al. 2011). Pine weevil is a very common cause of damage to planted seedlings in their first years in the field in Scandinavia, and mechanical site preparation is known to reduce the impact significantly (Örlander and Nilsson 1999; Petersson et al. 2005; Nordlander et al. 2011; Wallertz et al. 2018).

Our interpretation of the results is that the initial boost from AP treatment provides an advantage at establishment that is beneficial for survival of seedlings planted in mechanically-prepared planting positions but not in non-prepared soil. For second year growth, AP-treated seedlings seem to be able to utilize more favorable growing conditions i.e. a longer growing season for seedlings planted in capped mounds and mineral soil and a higher site index for seedlings planted in non-prepared soil.

The results presented here apply to sites with dry to mesic moisture classes on silty to coarse till, planted in spring/early summer. This study covers only initial establishment and early growth of the seedlings, and both the high variation in mortality and lack of general superior growth in capped mounds in our study could probably be a consequence of the very dry summer of 2018 and growth patterns might change over time. However, differences found between treatments at an early stage have been found to persist in the following years in other studies (Burton et al. 2000; Thiffault and Jobidon 2006) and a successful establishment is crucial for continued development of the newly planted seedlings (Brand 1991; Grossnickle 2000).

In this study, we have shown that AP treatment can enhance the establishment and early performance of planted Scots pine seedlings. Our results also indicated that seedlings planted in mineral soil are less sensitive to varying environmental conditions compared to seedlings planted in capped mounds. With the expectations of increasingly dry conditions in summer, we argue that the choice of main planting position for Scots pine needs to be adapted to site conditions.

Scots pine is most frequently planted on dry sites due to a relatively high drought hardiness compared to other species and is, therefore, the species that is most vulnerable to drought-induced damage. Variation in precipitation between years is generally large. Hence, there is always a risk of insufficient rainfall in the first weeks after planting for the seedlings to establish well on a certain site. Any site that is not classified as moist due to a near-surface groundwater supply could therefore be defined as potentially drought prone. According to our results, the preferred planting position of Scots pine at drought-prone sites is arguably an elevated position in mineral soil, as this is a safer choice regarding early survival. This argument is in line with other studies and reports that have concluded that planting in capped mounds should be avoided on drought-prone sites (e.g. Lammi (2006) and references therein).

Conclusion

A small addition of arginine phosphate at time of planting had a generally positive effect on the survival of *P. sylvestris* seedlings positioned both on capped mounds and in mineral soil following mechanical site preparation. In a year with low precipitation and high summer temperatures, like 2018, mineral soil appears to be the most appropriate planting position also in north Sweden. This result was supported by the positive relationship between survival and precipitation during the first 30 days following planting for seedlings positioned on capped mounds. The drawback of the mineral soil as a planting position is the low nutrient availability as, in contrast to the capped mounds, there is no decomposition of organic material supporting the establishing seedling with easily accessible nutrients. In this study, seedling growth in the mineral soil and on capped mounds did, however, not differ, but the AP treatment had a stronger positive effect on the growth of seedlings in mineral soil. Also, this positive growth effect increased with the length of the growing season. AP treatment had no significant effect on survival for seedlings planted in soils with no site preparation prior to planting and seedling survival using this planting method decreased as the length of the growing season increased. This negative correlation in part counteracted the positive effect of AP treatment on seedling growth that varied with the site index, that is, the more fertile the site, the more pronounced was the positive effect of AP treatment on seedling growth for seedlings planted in non-prepared soil.

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