Adapting Scots pine Regeneration to the Changing Climate

An investigation of the effects of seed coating, arginine addition, and planting position

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Cover: Possibly the bushiest Scots pine seedling in Sweden
(Photo: Matej Domevščik)

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Abstract

In Sweden, new forest stands have long been artificially regenerated using conifer seedlings. While standardized and widely adopted, this approach may need to be further adapted to the predicted increases in variations in the weather, such as extended periods of drought. In light of this, coated seeds of Scots pine, as well as adding arginine phosphate to the coating, were tested across 12 sites in Sweden. The coatings should facilitate germination and establishment of seedlings, while the addition of arginine phosphate would enhance growth. In another study across 11 sites in Sweden, the effects of arginine phosphate addition on survival and growth of nursery grown seedlings planted into mineral soil or in capped mounds were tested. Seedlings planted in mineral soil may be better adapted to dry conditions but may suffer from lower availability of nutrients compared to the capped mounds. The results showed no difference in survival between seedlings from coated seeds with or without arginine (following three growing seasons), whereas survival of nursery grown seedlings increased as a result of arginine addition (following two growing seasons). Arginine phosphate addition increased growth, both for coated seeds and for nursery grown seedlings planted in mineral soil as well as in capped mounds. The importance of precipitation was demonstrated in both studies, with positive relationships between survival and precipitation in the month following deployment. Hence, of the methods tested here, planting nursery grown seedlings in mineral soil with an amendment of arginine phosphate appears to be the most valuable to enhance both survival and growth of seedlings subjected to dry weather conditions.

Keywords: boreal forest, forest regeneration, coated seeds, planting position, Scots pine, arginine fertilizer, arginine phosphate, *Pinus sylvestris*.

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Föryngring av tall i ett förändrat klimat – studier av några metoder att förbättra resultatet

Abstract

I Sverige har nya skogsbestånd sedan länge artificiellt föryngrats med hjälp av plantor av barrträd. Även om detta tillvägagångssätt är standardiserat och allmänt antaget, kan det behöva anpassas ytterligare till de förutspådda ökningarna i vädervariationer, till exempel längre perioder med torrt väder. Mot bakgrund av detta testades inkapslade frön av tall samt tillsats av argininfosfat på 12 platser i Sverige. Beläggningarna ska underlätta groning och etablering av plantor, medan tillsats av argininfosfat skulle öka tillväxten. I en annan studie på 11 platser i Sverige testades effekterna av tillsats av argininfosfat på överlevnad och tillväxt av plantor som odlats i plantskolor placerade i mineraljord eller i täckta högar. Plantor placerade i mineraljord kan vara bättre anpassade till torra förhållanden men kan lida av lägre tillgång på näringsämnen jämfört med de täckta högarna. Resultaten visade ingen skillnad i överlevnad mellan plantor från belagda frön med eller utan arginin (efter tre växtsäsonger), medan överlevnaden för plantor som odlats i plantskolor ökade från tillsats av arginin (efter två växtsäsonger). Arginintillsats ökade tillväxten, både för frön från belagda frön och för plantor som odlats i plantskolor placerade i mineraljord såväl som i täckta högar. Vikten av nederbörd visades i båda studierna med positiva samband mellan överlevnad och nederbörd under månaden efter utplacering av belagda frön och plantering av plantor som odlats i plantskolor. Av de metoder som testas här förefaller plantering av plantor som odlats i plantskolor i mineraljord med tillsats av argininfosfat vara den mest lämpliga för att förbättra både överlevnad och tillväxt av plantor som utsätts för torra väderförhållanden.

Keywords: boreal skog, skogsföryngring, belagda frön, planteringsplats, tall, arginingödsel, argininfosfat, *Pinus sylvestris*

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Dedication

I dedicate this thesis to all the wonderful people I have met along the way.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. M. Domevscik, B. Häggström, H. Lim, J. Öhlund, A. Nordin. Large-scale assessment of artificially coated seeds for forest regeneration across Sweden (submitted manuscript).

II. B. Häggström, M. Domevscik, J. Öhlund, A. Nordin. (2021) Survival and growth of Scots pine (*Pinus sylvestris*) seedlings in north Sweden: effects of planting position and arginine-phosphate addition. *Scandinavian Journal of Forest Research*, vol. 36 (6), pp. 423–433.

Paper II is reproduced with the permission of the publishers.

The contribution of Matej Domevščik to the papers included in this thesis was as follows:

I. MD planned, organized, and performed the fieldwork by himself following a brief introduction to the already established field trials by the supervisors. MD was in charge of data and statistical analyses as well as writing the paper, with input from the co-authors.

II. MD planned and performed the fieldwork together with the main author. Further, MD assisted with data and statistical analyses and contributed to writing the manuscript.

Abbreviations

ANOVA	Analysis of variance
AP	Arginine phosphate (amino-acid based fertilizer)
CM	Capped mound
GLM	Generalized linear model
LM	Linear model
MS	Mineral soil
NPS	Non-prepared soil
SP	SeedPAD

1. Introduction

1.1 Studying boreal forest regeneration

Over the last century the forestry practice of clear-cutting has become established as the dominant forest management system in Sweden (Ostlund *et al.* 1997; Berg *et al.* 2008; Lundmark *et al.* 2013, 2021). Consequently, a need has emerged for efficient regeneration practices, since natural regeneration has not always been entirely successful. To support the practice, research on systematic regeneration started in the 20th century, as clearcut forestry was slowly gaining in importance (Lundmark *et al.* 2021). One of the first major publications on forest management in Sweden, particularly addressing regeneration, was "Skogsskötsel: handledning vid uppdragande, vård och föryngring av skog" (Forest management: guidance on the cultivation, care and rejuvenation of forests) by Anders Wahlgren (Wahlgren 1914). In this work, fundamental regeneration practices and systems were described, e.g., different planting positions, many of them still in use today.

Research around the mid-century focused on efficient and rational planting (Ebeling 1957) in line with the systematic and standardized clearcutting practice. Around the same time, forest selection programmes started to choose trees with desirable traits to be used in tree breeding programmes (Haapanen *et al.* 2015). Through a system of recurrent selection, breeding populations gradually improved with respect to selected traits such as survival, growth and resilience to pests and frost damage. This system is still in use today in seed orchards and provides seeds used in the regeneration process. In the 1960s, ploughing for forest regeneration was developed in Finland (Nyyssönen 1997) which added to the growing research on site preparation (Bärring 1967; Lundmark 1977). In the 1970s, several studies emerged on environmental factors effecting seedling establishment and mortality e.g. Söderström 1976 and Persson 1978. Around this time modern methods of soil preparation were also developed, alongside machines to perform them, such as mounders and disc trenchers (Örlander *et al.* 1990). Physiological studies on growth and establishment of seedlings continued into the 1980s (e.g. Örlander 1984; Ingestad 1987; Burdett 1990), with a focus on improving survival and growth.

Towards the end of the 20th century, the focus of research shifted as traditional studies striving for optimization of planting were complemented with studies on regeneration following natural disturbance dynamics (e.g. Attiwill 1994; Fries *et al.* 1997; Angelstam 1998). This was further advanced through studies on forest understory dynamics (e.g. Mallik 2003; Nilsson & Wardle 2005) and the role of fire in forest regeneration (e.g. Ryan 2002; Johnstone & Chapin 2006).

In recent years, the focus has been on genomic improvements to the seedling material (Lenz *et al.* 2020; Capador-Barreto *et al.* 2021), fuelled by decoding of the Norway spruce genome in 2013 (Nystedt *et al.* 2013). While the genome of the other dominant conifer species, Scots pine, still remains to be fully decoded (Pyhäjärvi *et al.* 2020), there are already studies using genotyping-by-sequencing to determine the relatedness and growth of Scots pine (Calleja-Rodriguez *et al.* 2019; Hall *et al.* 2020). Another major topic of research in recent times has been the study of climate change and its effects on forests (Lindner *et al.* 2010, 2014). This includes studies of pest damage to seedlings under warmer climate conditions (Nordlander *et al.* 2017), as well as the impact of extreme drought events on seedlings and trees (Lloret *et al.* 2012; Camarero *et al.* 2015).

1.2 Current practices and status

In the following section, current practices used in Swedish forest regeneration are briefly introduced.

1.2.1 Planting

The current Swedish Forestry model mainly consists of forest harvest by clearcutting, followed by soil scarification, and manual planting of nursery-grown seedlings of the native species Norway spruce and Scots pine (Figure 1) (Skogsstyrelsen 2020). This system is highly standardized and widely adopted, with approximately 84 % of clearcut areas in Sweden being regenerated by this method (Skogsstyrelsen 2019). The average rotation time for conifers is around 60-110 years, depending on site factors like latitude and site index (Skogsstyrelsen 2015).

Seeds used in the production of seedlings are collected from seed orchards specialized in producing high performing seeds. They are germinated in nurseries and grown under optimized conditions to become seedlings. For example, short-day treatment is applied to most conifer seedlings in Sweden, which stimulates them to produce the equivalent of a two-year old seedling in just one year (Rosvall-Åhnebrinkl 1982). Depending on the needs of the area, seedlings can spend several years in nurseries, producing a larger seedling with a more developed root system. In addition, seedlings planted particularly in southern Sweden are commonly provided with some form of mechanical protection in an effort to reduce the damage caused by pine weevils (*Hylobius abietis*), a common pest with significant economic consequences. These treatments can considerably increase investment per seedling as well as the resource requirements of nursery operations.

After they have reached a suitable size (usually around 12 cm), the seedlings are manually planted. Generally, growing containers filled with peat are used to separate seedlings and ensure ease of storage, transport and planting. Containers with a volume of either 30 cm^3 or 50 cm^3 are commonly used. Seedlings may also be freeze-stored for easier handling before planting.

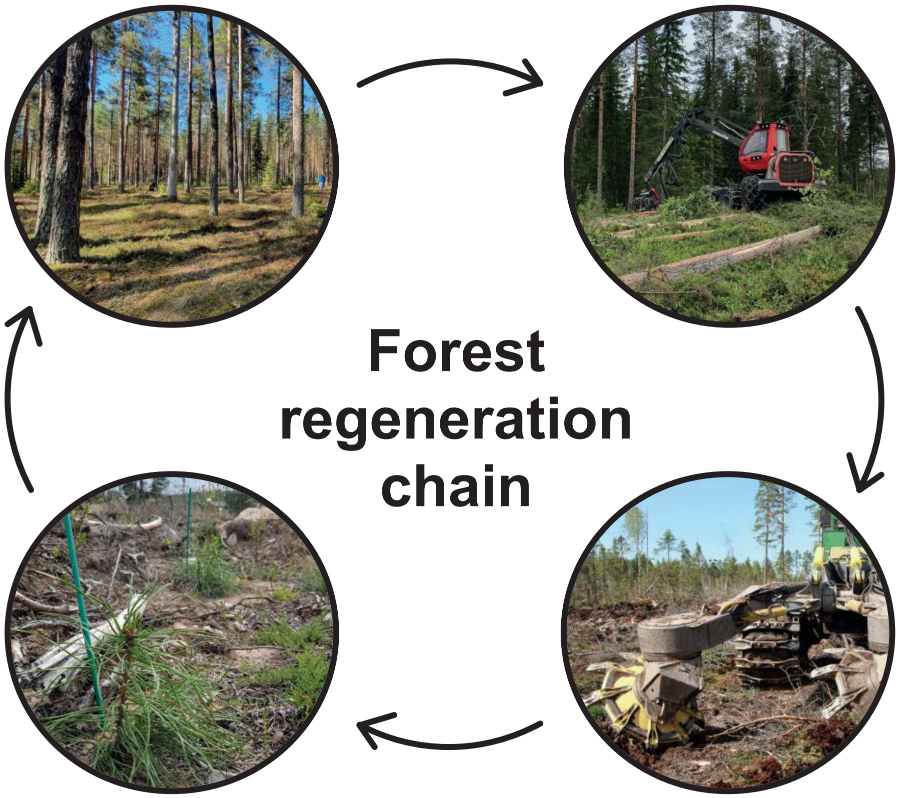


Figure 1. A representation of forest regeneration as commonly performed according to the current Swedish Forestry Model. Clockwise from top left: mature forest about to be harvested, forest harvest by clear-cutting, mechanical soil preparation, and planting of seedlings. Mechanical soil preparation picture by Jörgen Hajek, all other pictures by Matej Domevščik.

1.2.2 Natural regeneration

In contrast to artificial methods, natural regeneration relies on seed material from existing trees to regenerate the harvested area. While not a common method in the current Swedish Forestry model, it is increasingly discussed in connection with biodiversity conservation values (Brang *et al.* 2014). Natural regeneration was reportedly used on 4 % of the total regeneration area in Sweden in 2019 (Skogsstyrelsen 2019).

A commonly used method under this system is the seed tree method, where everything except for the seed trees is removed through a standard harvest. Afterwards, the regeneration site is prepared using machinery to create patches with good germination conditions. Seed trees then disperse their seeds, which readily germinate in the prepared patches, thus regenerating the stand. While seeds germinate over the whole stand, the most and largest are found in the prepared patches (Hagner 1962). When new seedlings average around 30 cm in height, the old seed trees are removed to provide space and reduce competition with the new stand. Another common method is the shelter wood system, where large mature stems from the old stand are left in the area, to provide seed material and shelter. The shelter wood system differs from the seed tree method mainly in the higher density of mature trees being left after harvest, to provide shelter for the regenerating stand. Both systems are, however, prone to storm damage, as the open stands that are created can suffer increased wind throw (Hånell & Ottosson-Löfvenius 1994). When selecting areas for natural regeneration, special attention needs to be paid to the occurrence of high winds as well as the type of trees left after harvest. In addition, inherent to the method itself, natural regeneration suffers from the inability to use genetically improved seed material (Simonsen 2013), a major factor in the current Swedish Forestry Model.

1.2.3 Direct seeding

Direct seeding refers to artificially regenerating a forest stand using bare seeds produced in seed orchards. Not all areas are suitable for this method, but when done well, direct seeding can result in dense stands of trees with well-developed root systems (Wennström 2001). While more common than natural regeneration, direct seeding is not a widespread regeneration method in the current Swedish forestry model. In 2019, direct seeding was used on 10% of the total regeneration area in Sweden (Skogsstyrelsen 2019).

Using seeds grown in seed orchards opens up the possibility of using genetically improved seed material as well as provenances from different regions. Both have been shown to increase survival and growth rates in planted seedlings (Persson 1994; Egbäck *et al.* 2017). Direct seeding is usually cheaper than planting nursery grown seedlings, but seeds need time to germinate and establish, which may increase rotation time. On the other hand, unlike planting nursery seedlings, direct seeding can be done immediately after scarification, thus compensating for the longer rotation time. Planting of nursery seedlings is commonly delayed for a few years after harvesting, as this has been shown to reduce the impact of pine weevils (Örlander *et al.* 1997; Normark & Sjölin 2015). Pine weevil damage peaks soon after harvesting and the insects target seedlings, but not seeds.

However, one challenge with direct seeding is the interannual variation in the quantity of seeds produced in orchards (Prescher *et al.* 2005), which can severely limit the availability of high quality seeds. In addition, seedlings from direct seeding have been shown to experience low establishment rates of around 20% (Palma & Laurance 2015; Grossnickle & Ivetić 2017) which can result in low restocking rates.

1.3 Climate adapted forest regeneration

Climate prediction models for Scandinavia forecast an increase in the overall precipitation and mean temperatures, but also consecutive drought days with no precipitation during the vegetation period (Christensen *et al.* 2001; May 2008; Chen *et al.* 2015). It is predicted that precipitation will become more variable and seasonal, with most of the increases during autumn and winter. On the other hand, according to some models, late spring and summer could experience more numerous and more extended periods of drought (Chen *et al.* 2015). Since most of the planting operations occur in late spring and early summer, we can expect more seedlings to struggle during the initial growing phase due to dry conditions. In the context of the predicted climate change, it thus appears important to adapt regeneration methods to ensure robustness under these more varied environmental conditions. Adequate soil moisture during the early growth phase is and will remain a core factor to consider. With increasingly variable precipitation, methods that increase seedling water acquisition and retention should be further explored and tested.

1.3.1 Coated seeds

One method that may improve performance under drier conditions is the use of coated seeds. As the name suggests, coated seeds consist of a single seed embedded in a layer of protective minerals. They represent a mid-way balance of investment between pre-growing seedlings in a nursery and direct seeding. Compared to direct seeding, seeds are somewhat protected from the elements by the coatings, while remaining cheap to produce and distribute. Importantly, coated seeds are intended to retain the benefits of direct seeding, such as lower risk of pine weevil damage, while increasing survival and decreasing the quantities of seeds needed. Studies show that while planted seedlings may have a high root biomass, naturally established seedlings have a much wider root distribution (Burdett *et al.* 1984; Burdett 1990). Similarly, Örlander 1984 demonstrated higher water potential in Scots pine seedlings established *in situ* compared to nursery planted ones for up to four years after planting. With the increasing possibility of drought, an extensive root system that ensures high water potential can translate into higher survival and growth rates (Grossnickle 2005). Further, significant moisture loss during periods of drought may also affect the survival of seedlings. In conifers, the majority of water loss occurs through transpiration via needles (Grier & Running 1977). Planted seedlings have developed considerable needle foliage in nurseries by the time they are planted (Figure 3), which facilities quick biomass acquisition through photosynthesis. In dry conditions where moisture retention is crucial, reduced needle surface may, however, offer benefits through lower moisture loss.

The idea of using coated seeds as a method of boreal forest regeneration is not new; however, so far very few results have been published following peer-review. Similar methods of seed distribution have been tried in the past, but none has yet been commercially successful or widely adopted. A notable example of a developed product from Sweden is the LandPuck™ system (Lappland Design AB Forest). The authors report using compressed peat, thought to help retain moisture, and burying the pads a few centimetres into the mineral soil layer. On their official website, Lappland Design AB Forest reports survival rates of up to 70% in the field and up to 93% in the laboratory, while promising a 30-40% reduction in cost compared to established methods. This is, however, based on unpublished self-reported data. Further, in a master's thesis examining LandPucks, the author reports an average of 70% survival after four years from 10 sites in northern Sweden (Wennström 2014).

1.3.2 Importance of planting position

Microsite conditions in different planting positions are a major source of variation in survival and growth of seedlings (Spittlehouse & Stathers 1990), largely as a product of local weather conditions. Therefore more frequent and longer periods with consecutive drought days in the future (Chen *et al.* 2015) suggest the focus should be put on analysing moisture retention of different planting positions. Currently, the two most widespread methods of mechanical soil preparation (scarification) in Sweden are mounding and disc trenching (Skogsstyrelsen 2018). Both of these methods invert the top layer of soil (Örlander *et al.* 1990; Sikström *et al.* 2020), creating planting positions of varying height and characteristics (see Figure 2). Lower planting positions are in the depression made by the soil preparation, directly in the mineral soil (MS). Higher planting positions are on top of the inverted material and comprise the upper mineral soil layer, a double layer of organic material and the underlying mineral soil; according to the definition of Sutton 1993, these are hereafter referred to as capped mounds (CM). The current general recommendation in Sweden is to plant on CM with the only exception in areas where the risk of drought is considered higher than normal (Skogsstyrelsen 2021). This recommendation may need to be adapted to future climate conditions.

The main benefits of planting into CM are increased availability of nutrients from the organic layer and a reduction in frost damage (Örlander *et al.* 1990).

On the other hand, planting in CM can lead to a higher risk of drought, especially on dry sites (Örlander *et al.* 1990). This may be connected to high variability in CM, from the perspective of preparation for subsequent planting (Sutton 1993; Söderbäck 2012). Since planting is mostly done manually, it is up to the planter to decide where exactly to plant. Ideally, seedlings planted in CM should be positioned as shown in Figure 2, with sufficient contact with the underlying mineral soil. This is not always easy, as a planting position classified as optimal from above may have a deep layer of organic matter underneath (see Figure 3). Such a barrier can significantly hamper survival and growth, as early contact with moist underlying soil is crucial (Burdett 1990; Grossnickle 2005). On sites with abundant woody debris or organic matter, planting in CM can potentially result in higher mortality, especially when combined with longer periods of drought.

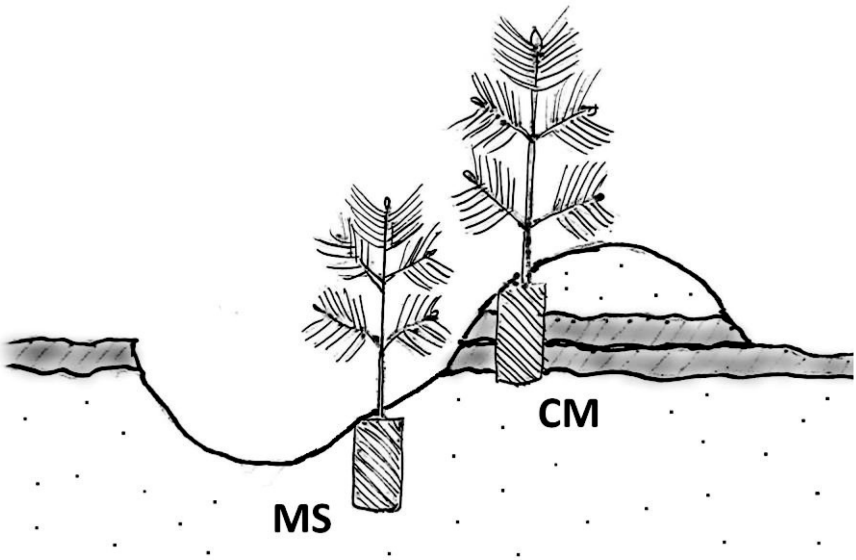


Figure 2. Cross section of the two common planting positions created by mechanical soil preparation through mounding or disc trenching. The shaded area represents the organic humus layer, while the speckled white area represents mineral soil. The two planting positions are referred to as mineral soil (MS) and capped mound (CM) (Sutton 1993) (drawing by Bodil Häggström).

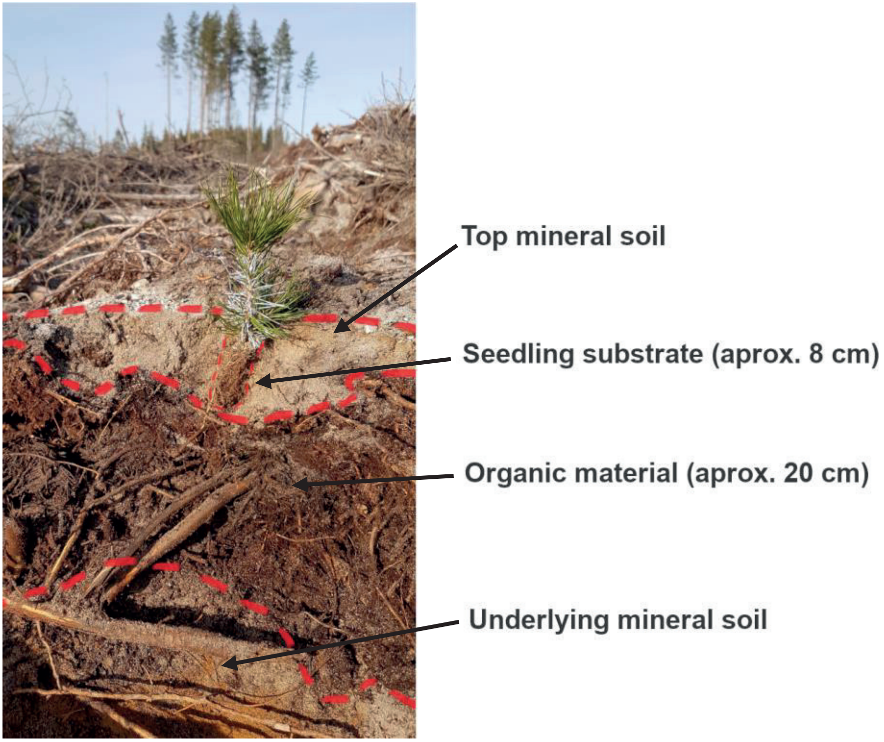


Figure 3. A cross section of a capped mound and underlying soil layers, with a containerized nursery grown Scots pine seedling planted at the top.

The main concerns when planting in MS include increased risk of frost damage and waterlogging, which can negatively affect the survival of seedlings (Örlander *et al.* 1990; Spittlehouse & Stathers 1990). However, the large latitudinal range and diversity of sites in Sweden means that not all sites are affected equally. On dry sites especially, planting in a high position in mineral soil may still result in higher survival overall. To this end, because of an average yearly rainfall of around 500-700 mm/m^2 (SMHI 2021), many of the sites in northern Sweden can already be considered dry compared to the south. As the climate changes in the future, the number of dry sites may increase further.

1.3.3 Nutrient amendments

With more unpredictable weather conditions at the time of planting, seedlings may struggle to survive the critical early growth stage. To address this issue, nutrient amendment to ensure vitality and growth may be considered. This is mainly done through fertilization with nitrogen, the limiting nutrient in boreal forests (e.g. Tamm 1991; Sponseller *et al.* 2016). Traditionally, nitrogen has been added to seedlings in its inorganic form as ammonium or nitrate (Juntunen & Rikala 2001). However, studies have shown that inorganic nitrogen can influence the root: shoot ratio of seedlings (Kaakinen *et al.* 2004; Hermans *et al.* 2006), resulting in higher investments in shoots at the expense of roots. It is important for seedlings to prioritize root growth early in the growing process, as the ability to acquire water effectively is critical to survival (Burdett 1990). By investing more in shoots early on, seedlings may reduce their ability to access sufficient water, which may lead to desiccation. This could be even further exacerbated in the future by the changing climate.

In recent years, fertilization with organic nitrogen fertilizer has emerged as a viable alternative to inorganic nitrogen (Wilson *et al.* 2013; Lim *et al.* 2021). Unlike inorganic forms of nitrogen, fertilization with organic nitrogen is associated with neither soil acidification nor increased risk of nitrogen leaching (Öhlund & Näsholm 2001, Hedwall et al. 2018). Importantly, organic fertilizer has been shown to increase root: shoot ratio in seedlings compared to traditional inorganic fertilizer (Cambui *et al.* 2011). Gruffman *et al.* 2012 found larger and more developed root systems as well as more fine roots in seedlings grown with organic arginine fertilizer, compared to conventional inorganic fertilizer. Having a larger root system early in the growing process may help seedlings to adapt to local soil moisture conditions, especially during periods of drought (Grossnickle 2005).

1.4 Aim

The aim of this licentiate thesis was to investigate the effect of planting position, arginine fertilization treatment and environmental variables on coated seeds and nursery-grown seedlings across 23 sites covering a range of latitudes in Sweden. The findings are considered in the context of a climate-changed future, with suggestions for future-proofing forest regeneration practices.

2. Methods

Methods presented here are a summary of those from the two papers included in this thesis, which I refer to throughout. For further details, please see those two papers, as well as the references provided.

2.1 Study sites

A total of 23 sites were established on clearcuts in Sweden between latitudes 58.7 °N and 67.1 °N, covering a latitudinal gradient of 1000 km (Figure 4). Paper I included 12 sites (Table 1) established in May-June 2017, while paper II included 11 sites established in May-June 2018 (Table 2). Due to the large latitudinal gradient, site establishment time had to be adapted to local growing conditions. The sites were chosen because of their dry characteristics, locations where Scots pine is commonly planted. Annual precipitation for all sites is between 500 and 700 mm/m^2, averaged over the 10-year period 2010-2019.

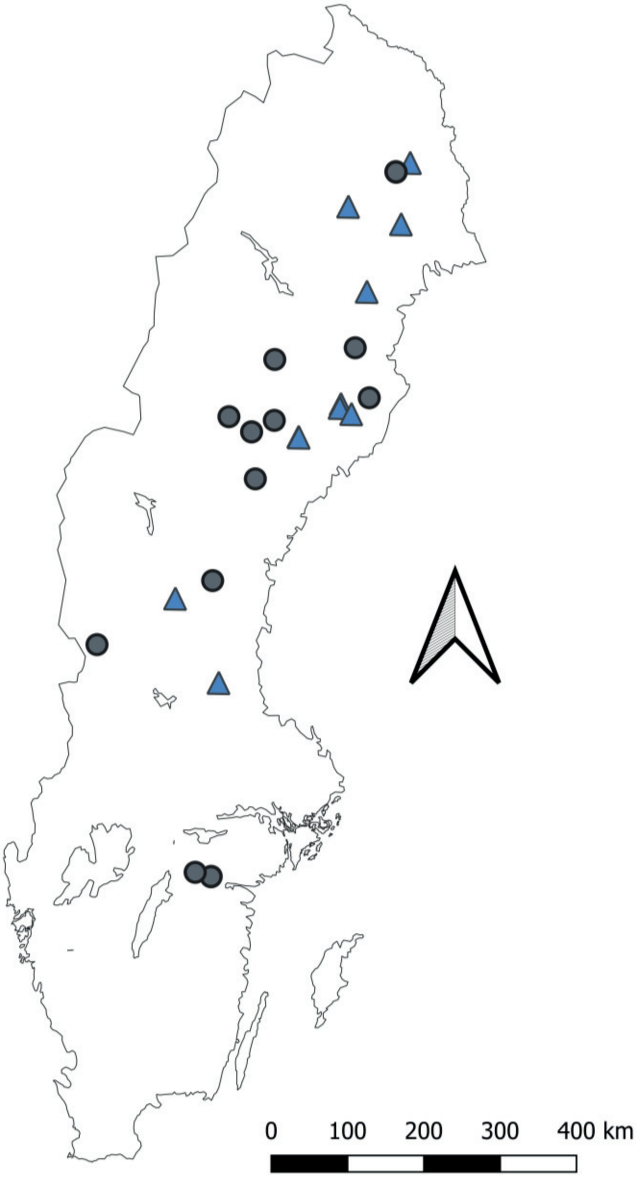


Figure 4. Map of the study sites across Sweden. Grey circles represent sites included in paper I, while blue triangles represent sites included in paper II.

Table 1. Description of the 12 experimental forest clear-cut sites from paper I, arranged from north to south.

Site name	Location (lat/long)	Elevation [m; a.s.l]	Growing season [days]	Topsoil composition	No. of replicated plots	
					SeedPAD unfertilized	SeedPAD fertilized
Rissavägen	66.99/22.13	241	139	Silty till	1	3
Nyback	64.95/20.44	143	170	Coarse till	6	2
Bergsvik	64.88/18.17	268	149	Coarse till	2	
Backmyran	64.35/20.71	175	170	Coarse till	5	7
Varpsjovägen	64.22/16.84	364	145	Coarse till	4	4
Tallsjö	64.14/17.92	319	149	Moist till	5	3
Stormon	64.03/17.44	297	145	Till	6	2
Svanatjarn	63.47/17.49	319	146	Till	4	2
Storulvsjö	62.28/16.3	418	146	Moist till	3	2
Nyhult	61.51/13.4	570	146	Sandy till		2
Lilla Malthult	58.8/15.77	69	194	Sandy till	6	2
Hostdagskärret	58.75/16.13	86	194	Podzol	6	2

Table 2. Description of the 11 experimental forest clear-cut sites from paper II, arranged from north to south.

Site name	Location (lat/long)	Elevation [m; a.s.l]	Growing season [days]	Precipitation first 30 days [mm]	Pot size (cm^3)	Number of measured seedlings
Pajala	67.09/22.30	200	130	13	30	288
Jokkmokk	66.64/20.30	260	130	48	50	107
Skajte	66.38/21.82	200	135	43	30	88
Kvällsberget	65.62/20.63	180	140	43	30	64
Hällnäs	64.31/19.64	300	145	44	50	68
Vindeln	64.26/19.61	180	145	45	50	81
Torrbergsknösen	64.18/19.91	180	145	44	50	14
Åselhål 2	63.95/18.44	280	145	24	50	62
Åselhål 1	63.95/18.45	260	145	24	50	42
Strömbacken	62.07/15.09	260	155	36	30/50	284
Källåsen	61.06/16.16	360	165	35	50	109

Table 3. Sum of precipitation in May-August 2017, 2018 and 2019 for all sites grouped into four clusters. Precipitation data were obtained from the SMHI open database using the weather station nearest to sites within each cluster (mean distance to sites 65 km, max distance 125 km). Precipitation sum values reported in mm.

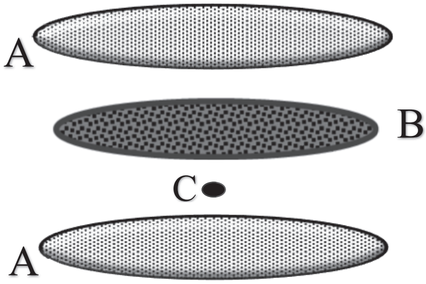
Cluster name	Range of latitudes	Number of sites	∑ precip. 2017	∑ precip. 2018	∑ precip. 2019
North Norrland	67.1-65.6	5	266.2	195.1	306.4
South Norrland	64.9-63.5	12	251	154.7	248.2
Mid-Sweden	62.3-61.1	4	325.7	239.2	211.9
Södermanland	58.8	2	187.6	134.4	228.1

2.2 Paper I

2.2.1 Material and data collection

The coated seeds used in this study were developed by Arevo and SweTree Technologies under the name of SeedPAD (SP). Each SP comprises a single seed of Scots pine (*Pinus sylvestris*), covered with a layer of vermiculate mineral, and wrapped in dissolvable polysaccharide foil (see Figure 4). The pads are 35 mm in dimeter with a thickness of 3.5 mm and deployed with the seed underneath. When exposed to sufficient water, the polysaccharide foil readily dissolves and thus vermiculate forms a seal over the seed, conserving moisture within. The SPs used were either fertilized with one dose of amino-acid based arginine-phosphate (AP - 10 mg N and 5.5 mg P) or unfertilized

In May-June 2017, both fertilized and unfertilized SPs were deployed in mineral soil exposed by disc trenching by the landowners, i.e., forest companies. Following commonly used procedures in Swedish forest regeneration, SPs were deployed in two experimental designs: circular and block. Circular plots were either marked as fertilized or unfertilized and contained 35 of the designated SPs in a circle with a radius of 5.64 (which forms an area of 100 m^2). Block plots comprised of two parallel rows of 50 m designated either fertilized or unfertilized with a SP every metre. Each SP in both designs was marked with a marking stick.



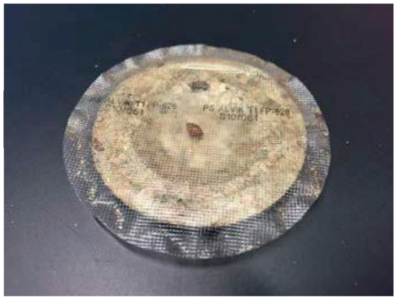


Figure 5. On the left, the composition of a SeedPAD: A – polysaccharide foil, B – layer of vermiculate, C – *P. sylvestris* seed. On the right, picture of an unfertilized SeedPAD version 5.0, used since 2016. The pads are deployed with the seed underneath.

Sites were surveyed in late summer 2018 and 2019, recording survival and growth of seedlings established from the SPs, considering seedlings within 10 cm of the marker stick to be the study seedlings. Growth of the live seedlings was measured as the distance from the ground to the top of the shoot, while sticks with dead or missing seedlings were considered non-surviving. In addition, in 2019 five seedlings from each treatment present at each site were carefully excavated for biomass analysis. Seedlings were dried for 24 hours at 60°C (to constant weight), then cut at the stem, and the root and shoot parts weighed separately.

At each site, topsoil data was classified using visual assessment. Climate data were downloaded from the open database of Swedish Meteorological and Hydrological Institute (SMHI 2021). For climate variables, data from the nearest available climate monitoring station for each site were used. The mean distance between sampling sites and climate stations was 32 km and the maximum distance was 46 km.

A controlled laboratory experiment was set up to investigate the water requirements of SPs, with the aim of identifying factors affecting attachment to the ground. A total of 135 SPs were used, separated into three different water addition rates combined with three different soil grain sizes. Soil was collected in 2020 from a clearcut in middle Sweden (Storulvsjö) while soil grain sizes were set to best represent field conditions. Water addition rates were determined using local precipitation data averaged for all sites. SPs were placed on soils with grain sizes of 1.7, 5.6 and 10.0 mm diameter to represent fine, medium, and coarse textured soil, respectively. Room temperature water (0.5 ml) was then added to the SPs every 1.5, 3 and 5 minutes for fast, medium, and slow treatment, respectively. Attachment was considered successful when the SP could not be easily pushed sidewise from the soil.

2.2.2 Analysis

To investigate the effect of fertilization, site and their interaction on survival, growth and biomass, a model III ANOVA (analysis of variance) was used with fertilization and site as variables. R-Studio software was used throughout the analysis (R Core Team 2019). To investigate survival, proportion of survival in each plot was used as the response variable, while growth and biomass were investigated using SP height and dry weight, respectively. Further, to find a subset of environmental variables that is significantly related to responses across site, a stepwise selection procedure was used. At each following step, a non-significant ($p>0.05$) variable was removed, and the new model was checked with Akaike Information Criteria (AIC) against the previous one. When values of AIC differed by less than 2, the model with fewer degrees of freedom was selected. After several steps this results in a final model which included only significant variables. Generalized linear models (GLM) were then used to determine which environmental variables had the most effect on survival of SPs. Variables included precipitation, temperature, wind, topsoil and vegetation period data.

Analysis of the data used in paper I had to be adjusted to local conditions. Because of a sampling error, three fertilized plots at Backmyran and two unfertilized plots at Lilla Malthut had to be excluded from analysis for 2018. Similarly, the two sites without both treatments present (Bergsvik and Nyhult) were excluded from ANOVA analysis.

2.3 Paper II

2.3.1 Data collection

A field experiment was set up in early summer 2018 to test the effects of fertilization and planting position on survival and growth of nursery seedlings. Seedlings were distributed in two treatment groups, fertilized with one dose of arginine-phosphate (AP - 40 mg N and 22 mg P) and unfertilized. Seedlings from both treatment groups were planted in one of the three planting positions: capped mounds (CM), mineral soil (MS), or non-prepared soil (NPS).

Two to four parallel blocks were set up at each of 11 sites in Sweden, between latitudes of 61.1 and 67.1 (see Figure 4). Each block comprised six parallel rows of approximately 20 seedlings, one row for each fertilizer/planting position combination. An exception to this was the northernmost site (Pajala), where 70-100 seedlings were planted per row. On each site the blocks were positioned adjacent to each other in relatively homogenous areas with no expected block effect, hence each site was treated as one plot in the analyses. Seedling material and size (30 cc and 50 cc pots) varied between sites, depending on the site owner (Table 2).

Fieldwork was performed following the second growing season, in August-September 2019. Seedlings next to a marking stick that had green needles were considered to have survived, whereas dead or missing seedlings were recorded as non-surviving. Every second surviving and undamaged seedling in a row, after a random start at first or second seedling, was also measured for leader shoot length i.e., height between top branches and terminal bud.

2.3.2 Analysis

ANOVA was performed on survival and growth data using R-Studio software (R Core Team 2019). To investigate survival, survival log-odds (ratio of the probability of survival to probability of death) were used as the response variable, while growth was investigated using leader shoot length. The explanatory factors for both models were site, planting position and fertilization treatment. Model III ANOVA was used to detect any interaction between the main factors and followed up with model II ANOVA in cases where there was no interaction. In addition, linear models (LM) and generalized linear models (GLM) were used to test which environmental variables had the most effect on survival, these included precipitation in the first 30 days after planting, length of growing season and site index.

Analysis of the data used in paper II had to be adjusted to local site conditions. Torrbergsknösen was excluded from the growth measurement analysis for all positions due to the high number of damaged seedlings. Similarly, Källåsen was excluded from the analysis of NNS position for growth due to very low survival. Åsehål 1 was excluded from both survival and growth analysis of the NPS position since this position was not used at this site. At Strömbacken, half of the 50 cc and all of the 30 cc seedlings were harvested and measured prior to inventory. The two datasets were combined prior to analysis.

3. Results

Results presented here are a summary of those from the two papers included in this thesis, which I refer to throughout. For further details, please see those two papers, as well as the references provided.

3.1 Paper I

Three growing seasons after deployment, the average establishment rate of SeedPADs was 55% and 58% for fertilized and unfertilized variants, respectively (Table 4). The establishment rate ranged between 22% and 87% across 12 sites, and there was a significant effect of site on establishment rate (Table 5). Similarly, site also had a significant effect on height after second and third growing season and on biomass after third growing season (Table 5). Arginine phosphate fertilization had no effect on establishment rate for either of the years, while for height and biomass it was site dependent, as seen in the significant interaction between site and fertilization after three growing seasons (Table 5).

Table 4. Average survival, height and biomass of seedlings established from the SeedPADs. Surveys were performed in autumn 2018 and 2019. Estimated marginal mean values ± SE.

	Growing seasons after deployment	**SeedPAD unfertilized**	**SeedPAD fertilized**
Survival (%)	2	53 ± 0.01	46 ± 0.01
Survival (%)	3	58 ± 0.01	55 ± 0.01
Height [cm]	2	7.0 ± 0.6	6.8 ± 0.5
Height [cm]	3	14.9 ± 0.4	14.6 ± 0.5
Total biomass [g]	3	4.8 ± 0.9	8.8 ± 0.9
Shoot biomass [g]	3	4.0 ± 0.8	7.7 ± 0.8
Root biomass [g]	3	0.7 ± 0.1	1.1 ± 0.1
Root: shoot ratio	3	0.18	0.14

Table 5. Results (F- and p-values) from two-way ANOVA examining the effect of fertilization treatment (AP), site and their interaction on SeedPAD survival, height and biomass. Significant effects (p<0.05) are highlighted in bold.

	Growing seasons after deployment	**Treatment**	**Site**	**Interaction**
Survival	2	F(1,46)=2.943, p=0.09	F(9,46)=3.648, **p=0.001**	F(9,46)=0.822, p=0.59
Survival	3	F(1,51)=0.526, p=0.47	F(9,51)=4.028, **p<0.001**	F(9,51)=1.168, p=0.33
Height	2	F(1,28)=0.367, p=0.54	F(9,28)=3.003, **p=0.01**	F(9,28)=0.408, p=0.91
Height	3	F(1,324)=0.227, p=0.63	F(9,324)=34.849, **p<0.001**	F(9,324)=7.029, **p<0.001**
Total biomass	3	F(1,93)=0.055, p=0.81	F(9,93)=55.753, **p<0.001**	F(9,93)=5.740, **p<0.001**
Shoot biomass	3	F(1, 93)=0.060, p=0.01	F(9, 93)=55.822, **p<0.001**	F(9, 93)=6.414, **p<0.001**
Root biomass	3	F(1, 93)=0.027, p=0.13	F(11, 93)=51.710, **p<0.001**	F(9, 93)=2.201, **p=0.02**

Despite the non-significant effect of fertilization on seedling height, shoot biomass was on average 93% higher for fertilized than unfertilized SPs three growing seasons after deployment (Table 4). Similarly, root biomass was on average 84% higher for fertilized compared to unfertilized SPs. A significant interaction between fertilization and site indicates that effect of fertilization varied between sites (Table 5). Further investigation revealed positive significant interaction between fertilization and length of growing season ($F(1,109)=6.121$, $p=0.01$)

Using linear regression modelling of environmental factors collected for our sites, two weather conditions were shown to have a significant effect on survival of SPs. Maximum wind speed and maximum precipitation in the first six weeks after deployment both had a significant effect on the rate of seedling survival. High wind speed decreased survival by 9% and 6%, for fertilized and unfertilized SPs, per each additional m/s (Figure 7A). On the other hand, precipitation increased survival of both SP treatments by 11% per each additional 10 mm of precipitation (Figure 7B).

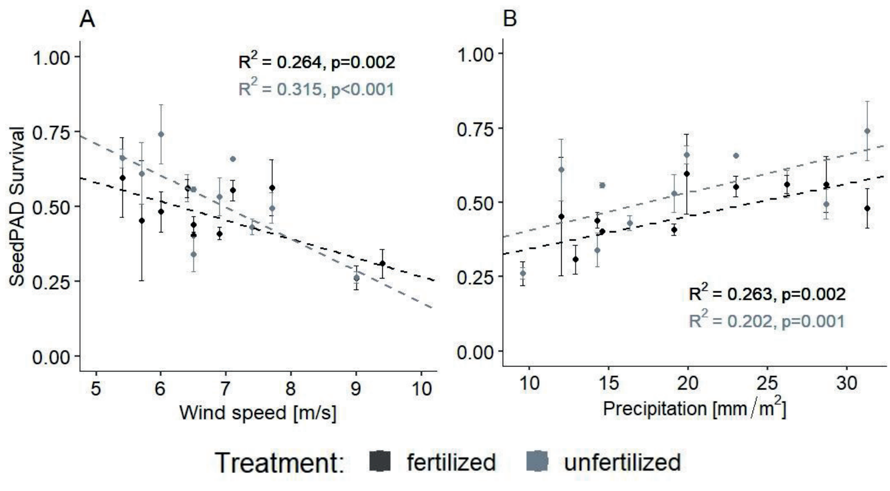


Figure 7. Effect of maximum wind speed (A) and maximum precipitation in a single day (B) within six weeks of SeedPAD (SP) deployment in early summer 2017 at 12 clear-cut forest sites on survival of seedlings from unfertilized and fertilized SPs in late summer 2018. Black lines and circles denote fertilized SPs, while grey ones unfertilized SPs.

The results of laboratory testing of SPs indicated that availability of sufficient moisture plays a key role in proper attachment. Two clear trends can be seen in Figure 8, increasing speed of water addition reduces the water requirement overall, as does the smaller soil grain size. On average, SPs on coarse grained soil required 34% more water than on fine grained soil. Similarly, the increase in water requirement needed for dissolution between slow and fast treatment within a soil texture type was c. 30%.

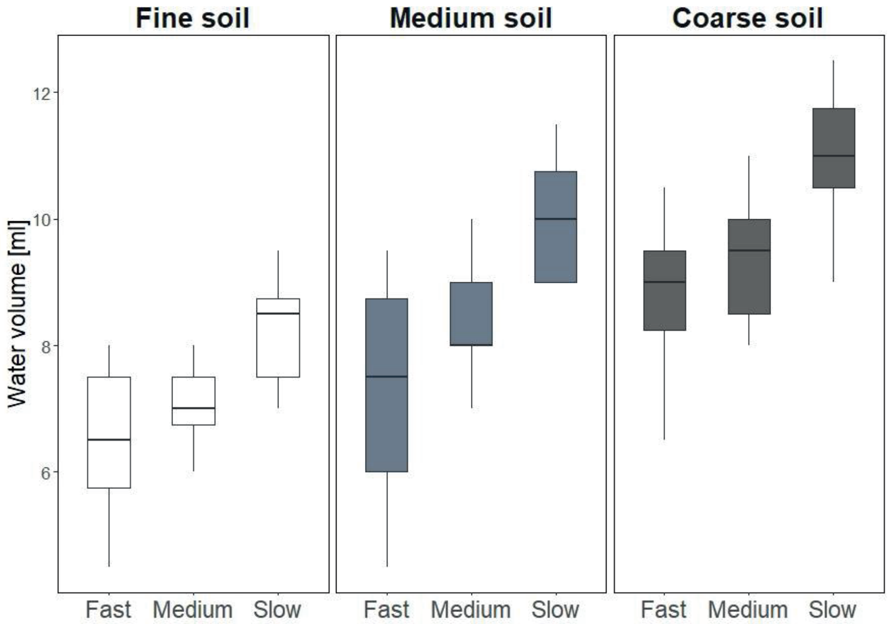


Figure 8. Water volume required for dissolution of SeedPADs, compared across three different soil mixtures and speeds of water addition. For soil treatments, grain sizes were set to 1.7, 5.6 and 10.0 mm diameter for fine, medium, and coarse soil, respectively. For speed of treatments, SPs had water dripped onto them every 1.5, 3 and 5 minutes for fast, medium, and slow treatments, respectively.

3.2 Paper II

Following two growing seasons in the field, the nursery grown seedlings fertilized with arginine-phosphate exhibited significantly higher survival, irrespective of site and planting position (Table 6, Figure 9A). The effect of planting position on seedling survival exhibited an interaction with site, i.e., the positive effect of planting into capped mounds varied between sites (Table 6). Variation in survival between sites was higher for CM compared to MS, but this turned out to be highly dependent on precipitation in the first 30 days after planting. Survival of seedlings in CM increased significantly with more precipitation (Figure 9C), while the opposite was true for seedlings planted in MS. The latter relationship was weaker, but still significant and explains 25% of the variation in survival, compared to 52% for seedlings planted in CM. In addition, at sites with little precipitation, the differences between survival in CM and MS were higher, highlighting the greater drought resilience when planting in MS.

There was a positive effect of fertilization on seedling growth, which was larger for MS than CM (Table 7, Figure 10A). In addition, the effect of fertilization increased with the length of growing season, especially for the seedlings in MS (Figure 10 B & C).

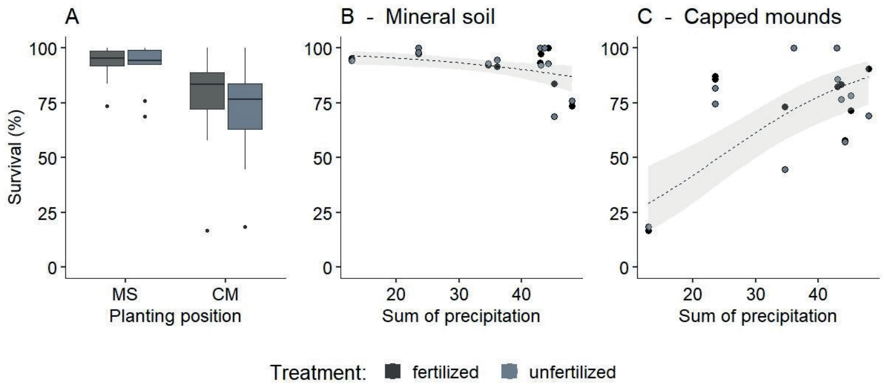


Figure 9. The effect of planting into mineral soil (MS) and capped mounds (CM) on survival (A) of nursery seedlings. The effect of sum of precipitation [mm/m^2] in the first 30 days after planting on survival of seedlings planted into mineral soil (B) and capped mounds (C).

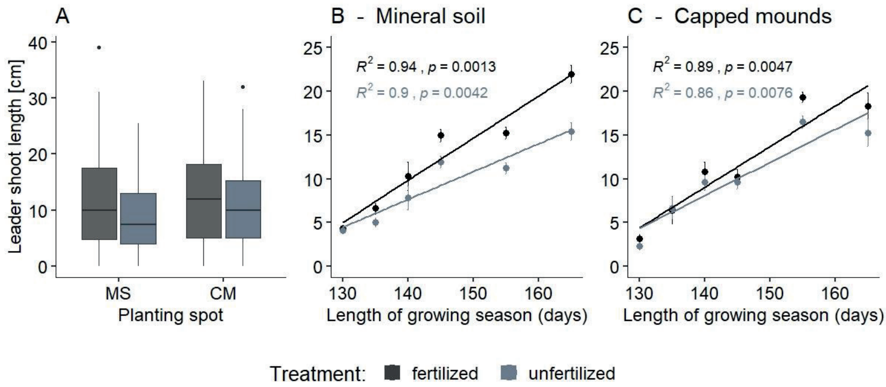


Figure 10. The effect of planting into mineral soil (MS) and capped mounds (CM) on length of leader shoot (A) of nursery seedlings. The effect of length of growing season in days on the length of leader shoot of seedlings planted into mineral soil (B) and capped mounds (C).

The survival of seedlings planted in positions that had not been mechanically prepared (NPS) was, on average, 58%. There was no significant effect of fertilizer on survival in this planting position. Instead, there was a negative relationship between survival and length of growing season, i.e., higher seedling survival in areas with shorter growing seasons. Site index was the most important variable explaining growth. Further, a positive fertilizer effect on seedling growth was found, which was more pronounced on sites with a higher site index (Paper II, Table 5A)

Table 6. Results from ANOVA examining the effects of site, fertilization treatment (AP), planting position and interaction between site and position on nursery seedling survival following two growing seasons in the field. Significant effects (p<0.05) are highlighted in bold.

	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**

Table 7. Results from ANOVA examining the effect of site, fertilization treatment (AP), planting position and their interaction on leader shoot length of nursery seedlings following two growing seasons in the field. Significant effects (p<0.05) are highlighted in bold.

	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	**0.03**
Site x Treatment x Position	84	10	0.45	0.92
Residuals	14955	805		

4. Discussion

4.1 Coated seeds

With an average survival rate of 56% across all sites three growing seasons after deployment, the survival rate of coated seeds, i.e. SeedPADs (SPs), was between what is commonly reported for direct seeding (c. 20%) (Wennström *et al.* 1999) and planting of nursery-grown seedlings (c. 75%) (Hjelm *et al.* 2019; Sikström *et al.* 2020). In the present study, the landowners of the different sites performed deployment of coated seeds and hence the survival rates may better reflect the normally occurring variabilities connected with commercial planting operations than studies on single sites undertaken by a single deployment team. In addition, in the region in which most of the sites are situated – the southern part of Norrland – the summer season of 2018 was exceptionally dry (Table 3), i.e., seedlings grown from SPs in this region experienced severe drought conditions one year after establishment, further increasing the range of environmental variability covered by this study.

Perhaps not surprisingly, therefore, the variation in survival was largely explained by site-specific factors tied to soil moisture, namely precipitation and wind speed. The study indicates that SPs benefit from a heavy precipitation event in the first six weeks after deployment, as seen by the significant positive relationship between maximum precipitation and survival (Paper I). The importance of precipitation early in the establishment phase for survival of nursery seedlings has been reported previously (Sukhbaatar *et al.* 2020), including in Paper II appended to this thesis. However, in the case of coated seeds, proper attachment to the ground is a unique first step not faced by planted nursery seedlings. The results of Paper I indicate that precipitation may have enhanced attachment and, in turn, survival of the emerging seedling, which requires good contact between roots and available soil water early in the growth process (Burdett 1990; Grossnickle 2005). Although the polysaccharide layer was designed to readily dissolve in contact with water and facilitate attachment, local precipitation conditions may have been insufficient for all the SPs to attach properly. This is supported by results of the dissolution experiment, where a volume of up to c. 12 ml of water was required for a SP to attach properly on coarse soil (Figure 8). SeedPADs not properly attached may stay slightly suspended above the ground and thus lack a direct connection with the soil water. In addition, there was a significant negative effect of maximum wind speed on survival of SPs (Paper I), suggesting a risk of mechanical removal of SPs and/or desiccation of the growing environment of the emerging seedling.

While seeds are largely resistant to drought prior to germination, once the seed coat splits and plant organs are exposed, young seedlings are highly vulnerable to environmental factors (Bewley & Black 1994). At this early stage, maintaining sufficient water potential is critical for establishment and continued growth (Burdett 1990). Covering the seeds with a protective vermiculate layer, as described for the SPs in Paper I, can reduce variations in temperature and moisture found on the surface of mineral soil (Evans & Young 1972; Oleskog & Sahlén 2000; Winsa 2016). With an average survival rate of c. 56%, it is clear that SP seeds can successfully germinate and develop into vital seedlings under field conditions. Moreover, survival data collected after three growing seasons (Table 4) shows that once established, seedlings were able to survive even severe drought, i.e., the summer of 2018.

Yearly precipitation values in northern Sweden are relatively low at around 500-700 mm/m^2 (SMHI 2021) and precipitation falls mainly outside the recommended early summer planting window. With predictions of increased drought in the future (Christensen *et al.* 2001; Chen *et al.* 2015), good contact with the underlying soil water will further increase in importance. Seed coatings may offer advantages for successful soil attachment of seeds, and high variation in early summer precipitation may be counteracted by watering immediately after SP deployment. The volume of water required for each SP is small when there is such targeted watering. Watering would facilitate the critical attachment step and reduce the variability in survival associated with it. Further, the small and uniform form of SPs, coupled with the fact that they do not need to be dug in mean that there is the potential for large-scale mechanization, including watering at deployment. This scalable approach may also offer other benefits, such as reducing the impact of future labour shortages (Rantala *et al.* 2009; Rantala & Laine 2010) and lowering silvicultural costs when deployed at scale. Another possible solution for small-scale private forest owners, who may be more flexible in their operations than forestry companies, is to perform SP deployment right before a large, forecasted precipitation event. Such adaptable, short notice operations are made possible through easier handling, transport, and storage of SPs compared to nursery seedlings. Seeds within the pads remain dormant until they are exposed to sufficient water (Bergsten 1987; Bewley & Black 1994), thus greater control over the start of germination is possible. In this way, increased storability of SPs could also decrease waste in planting operations, which can occur if seedlings are not planted within a given time frame after leaving the nursery.

4.2 Fertilization with arginine phosphate

The addition of arginine-phosphate (AP) fertilizer to the seed coating had no significant effect on the survival of seedlings emerging from SPs following three vegetation periods in the field (Paper I). This is in contrast to a study by Castro *et al.* 2021 performed at one of the sites also included in the current study (i.e. Paper I), who found a 50% increase in survival rate after one growing season as a result of adding nitrogen fertilizer, either as arginine phosphate or as mineral ammonium nitrate. However, at this particular site, the addition of AP also increased survival of the SPs in the experiment described in Paper I by 14% after two growing seasons. The apparent site dependency of the AP effect on the seedlings from SPs highlights the interaction between fertilizing and other environmental variables. In contrast, for the experiment using nursery grown seedlings presented in Paper II, there was a positive effect of AP addition irrespective of site and planting position.

The AP fertilizer did, however, enhance seedling growth in the current study as well as in the study by Castro *et al.* (2021). The total dry mass of seedlings from fertilized SPs was, on average across all sites, 83 % larger than that of seedlings from unfertilized SPs (Paper I). For seedlings in the work presented in Paper II, the AP enhancement of growth (measured as leader shoot length) was 13% for seedlings positioned in capped mounds (CM) and 29% for those in mineral soil (MS). While the positive effect of arginine addition on seedling growth is well documented (Cambui *et al.* 2011; Gruffman *et al.* 2012; Wilson *et al.* 2013; Lim *et al.* 2021), the positive effect of arginine on survival of Scots pine seedlings appears to be more rarely reported. This may be because most studies on arginine fertilization do not focus on survival, but instead focus on its positive effects through enhanced root growth and increased mycorrhizal infection of roots. For instance, Gruffman *et al.* 2012 reported positive effects from AP addition on growth, but no significant effect of arginine on survival of Scots pine seedlings compared to commercial inorganic fertilizer addition at three sites in northern Sweden.

In the study presented in Paper II, increased survival of nursery grown seedlings was indeed probably tied to the AP fertilizer's positive effect on root growth and mycorrhizal colonization (Gruffman *et al.* 2012). Both have been shown to increase water uptake capacity by seedlings, a crucial factor in early stage development (Grossnickle 2005; Brunner *et al.* 2015). In this case, it may be that AP increased early growth of roots and thus helped nursery seedlings survive the immediate summer drought of 2018. This is further supported by the fact that the positive effect of AP on growth was higher in the drought prone CM planting position than in the MS position. In comparison, SPs experienced drought conditions during the second year after planting, making it harder to see the effect of drought on survival. In addition, it seems possible that any AP-facilitated survival benefits of SPs may have been subordinate to proper attachment.

While not increasing survival, AP addition did increase root growth of SPs, resulting in 84% higher root biomass (Paper I), although this was site dependant. Previous studies indicate that high soil retention of arginine may stimulate seedlings' investment in roots to increase soil exploration (Inselsbacher & Näsholm 2012; Lim *et al.* 2021). In this case, it seems that AP addition to SPs further enhanced the already wide root distribution found in seedlings grown directly from seeds in field conditions (Burdett 1990). This may have contributed to sufficient water uptake rates for the properly attached SPs to survive the 2018 summer drought. A key factor to consider here is the high soil retention of arginine, which is also important from the perspective of reduced nitrogen leaching. The MS planting position, in particular, is considered more at risk of waterlogging (Örlander *et al.* 1990), where water can saturate the soil and increase nitrogen leaching. This is commonly observed with inorganic fertilizers after soil preparation like disc trenching (Rappe George *et al.* 2017). In contrast, in our study, high soil retention of arginine may have facilitated the continuous supply of nutrients to both nursery seedlings and SPs. Considering the increased variability with extreme precipitation events predicted for the future (Nikulin *et al.* 2011; Chen *et al.* 2015), arginine may be better suited for seedling fertilization, particularly for the MS planting position

Predictions of future climate in Scandinavia also show increased occurrence of drought periods, as occurred in the summer of 2018, as well as increases in mean temperature (Christensen *et al.* 2001; Chen *et al.* 2015). Under such circumstances, AP-facilitated root growth can lead to increased water uptake, that may, in turn, enhance survival rates of seedlings. Further, with the increasing mean temperature, we can expect an extension of the growing season, especially in the north. This would further increase the effectiveness of AP treatment on leader shoot growth of nursery seedlings, particularly for the more drought resilient MS planting position (Paper II).

4.3 The choice of planting position

Data on survival of the nursery grown seedlings from sites with very low precipitation during the first month after planting suggest that while seedlings planted in CM are more sensitive to drought, those planted in MS may suffer from high precipitation. However, the negative effect associated with MS explained less of the variation in survival than the positive effect did for CM. This suggests that the negative trend in MS might be better explained by other variables, such as increased frost damage. Nevertheless, it makes sense if we look at the shapes of the two planting positions (Figure 2). CM seedlings are planted on a raised mound, where water can quickly flow away, while MS seedlings are planted in a depression where water can collect and saturate the soil (Örlander *et al.* 1990). Still, the largest difference in survival between the two planting positions was observed on sites with lower precipitation during the initial establishment period. Together with higher survival in MS than CM, this suggests that drought is the most important factor.

Alongside precipitation, there are other important factors influencing seedling establishment, such as soil scarification, plant material, and quality of planting performance (Sikström et al. 2020). When creating capped mounds, the presence of a large amount of logging residue leads to formation of rough pockets of organic material, leading to poor contact with the mineral soil below. The only site in the dataset that had significantly higher survival and growth for seedlings planted in CM (Strömbacken) had a relatively small amount of logging residue, while not being unique in relation to any other site features. This suggests that Strömbacken may have had higher quality capped mounds, which led to greater survival and growth of seedlings planted in CM.

Research shows that some mechanical site preparation methods can affect up to 90% of all plant cover on clearcuts (Eriksson & Raunistola 1990) as deep and wide trenches are made to remove the top humus soil. This highly invasive procedure can have long lasting impacts on plant cover (Eriksson & Raunistola 1990), water quality (Marketta 1992) and species composition (Bergstedt et al. 2008). As predictions of increased drought periods highlight the more drought resistant MS planting position, novel methods of soil scarification can be considered. Currently, all the humus and a portion of the mineral soil layer are inverted to create the CM position. If the goal is to plant only in MS, perhaps a less invasive scarification method can be used instead. By only removing a thin and shallow strip of the humus layer and keeping the position flat, damage to seedlings and soil may be reduced, while keeping the benefits of the MS position. Combining several steps into a single process, such as scarifying and mechanized deployment of coated seeds, could further reduce impacts on the soil by reducing the amount of disturbance caused by repeatedly driving over the site with large machinery.

5. Conclusion

The results presented here have the potential to help adapt forest regeneration methods to the climate-changed future. The use of coated seeds, potentially combined with watering at deployment, may turn out to be mechanizable, thus facilitate regeneration and decrease negative impacts of today's extensive soil scarification. Furthermore, the addition of arginine phosphate can increase survival of planted nursery-grown seedlings and promote growth of both nursery-grown seedlings and seedlings from coated seeds. Such benefits may be particularly useful when planting nursery seedlings directly into mineral soil, where reduced variation in survival was recorded compared to planting in capped mounds.

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Popular science summary

Climate change is increasingly affecting weather patterns around the world, with increased rainfall intensity and temperatures. For Scandinavia, models predict more and longer periods of drought, especially during the summer. This could have a negative effect on forest regeneration, as the majority of new seedlings are planted in early summer. If seedlings do not receive enough water during the first few weeks, they might experience problems with growth or even die. To prevent this, we need to adapt the current system of forest regeneration to better fit with these new climate conditions.

In this research I tested how different factors affect survival and growth of Scots pine (*Pinus sylvestris*) growing on clearcuts in relation to climate change. First, I tested coated seeds, which are seeds wrapped in protective coating (see Figure 5) that are deployed on clearcuts. In this way, the seed is somewhat protected from the elements, but grows soon after creation of the clearcut. I found that the survival of such seeds after three seasons is around 56% and depends greatly on rainfall in the first month after deployment. Second, I tested different planting positions for seedlings within clearcuts, because this may have a big impact on how much water the seedlings get. In this case I compared the two most common positions: planting on top of inverted soil and planting directly into the mineral soil (see Figure 2). I found that in dry areas, it is better to plant directly into mineral soil, as the chance of survival is around 8% better. With predictions of increased drought, planting in mineral soil should become more common. Third, adding organic fertilizer increased biomass of seedlings growing from coated seeds, while in nursery-grown seedlings it increased growth and survival. So, by increasing root growth, organic fertilizer may help seedlings establish and form a good connection with the soil water underneath.

Populärvetenskaplig sammanfattning

Klimatförändringarna påverkar i allt högre grad vädermönster runt om i världen, som ökad regnintensitet och temperaturer. För Skandinavien förutspår modeller fler och längre perioder av torka, särskilt under sommaren. Detta kan ha en negativ effekt på skogsföryngringen, eftersom en majoritet av nya plantor planteras under försommaren. Om plantorna inte får tillräckligt med vatten under de första veckorna kan de få problem med tillväxten eller till och med dö. För att förhindra detta måste vi anpassa det nuvarande systemet för skogsföryngring för att bättre passa dessa nya klimatförhållanden.

I denna avhandling testade jag hur olika faktorer påverkar överlevnad och tillväxt av tall (*Pinus sylvestris*) som växer på kalhyggen i relation till klimatförändringar. Först testade jag inkapslade frön, som är en regenereringsmetod där ett frö lindas in i skyddande beläggning (se Figur 5). På så sätt skyddas fröet något från väder och vind, men växer redan från början på kalhyggen. Jag fann att överlevnaden för sådana frön efter tre säsonger är cirka 56% och beror mycket på nederbörden under den första månaden efter utplaceringen. För det andra testade jag olika placeringar av planterade plantor inom kalhyggen, eftersom det kan ha stor inverkan på hur mycket vatten plantorna får. I det här fallet jämförde jag de två vanligaste positionerna: plantering ovanpå omvänd jord och direkt i mineraljorden (se Figur 2). Jag upptäckte att i torra områden är det bättre att plantera direkt i mineraljord, eftersom chansen att överleva är runt 8% bättre. För det tredje ökade tillsatsen av organisk gödning biomassan i belagda frön, medan det i plantskolor ökade tillväxten och överlevnaden. Som sådan kan organisk gödning hjälpa plantor att etablera och bilda en god förbindelse med markvattnet under, genom att öka rottillväxten.

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II

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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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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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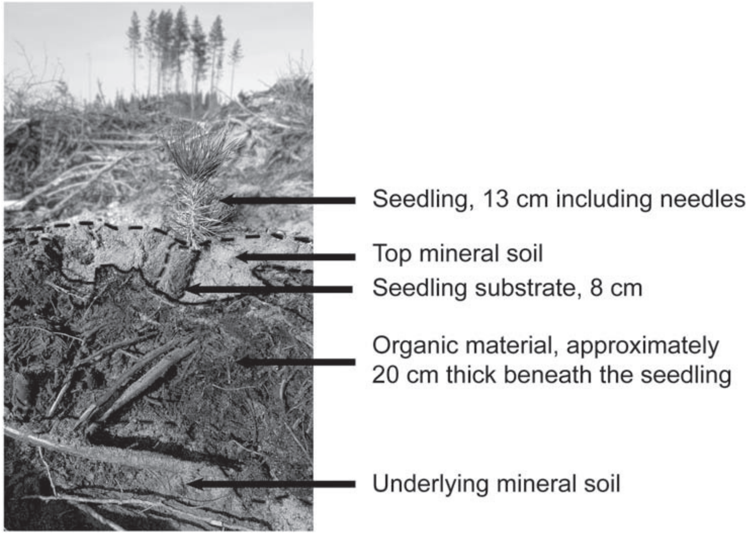

Figure 1. A cross section of a capped mound that would be classified as optimal when looking from above ground. The cross section reveals that the planting position is not optimal for the seedling to reach capillary water. Dashed lines mark the top surface of the capped mound, top and bottom of the organic 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**

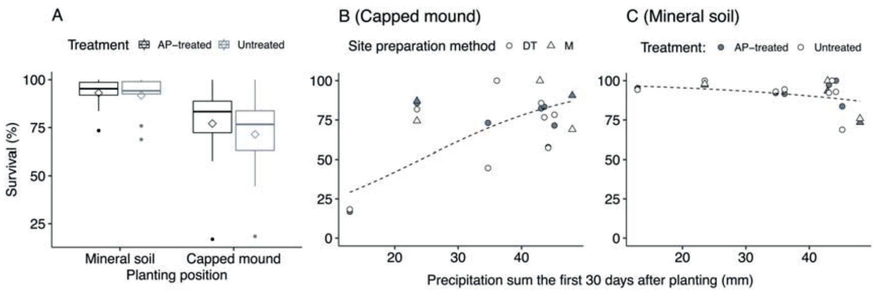

Figure 3. (A) Box and whisker plots of the observed proportional survival range of seedlings planted in mineral soil and on capped mounds with (black) or without (gray) arginine phosphate (AP) treatment across eleven study sites along a north-south gradient in northern Sweden. The diamonds indicate mean 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.

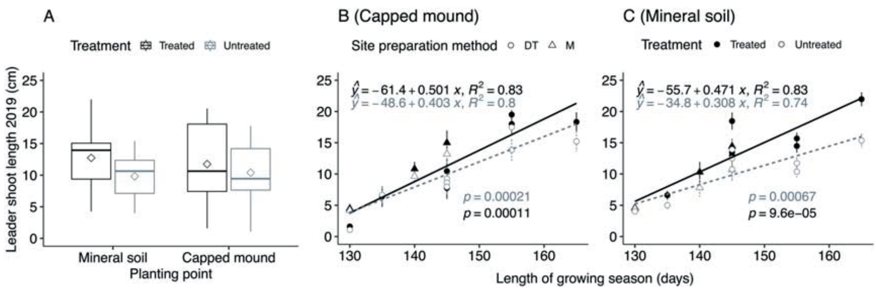

Figure 4. (A) Box and whisker plots of the range of observed mean leader shoot length values of seedlings planted in mineral soil and on capped mounds with (black) or without (gray) arginine phosphate (AP) treatment across eleven study sites along a north-south gradient in northern Sweden. The diamonds 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)).

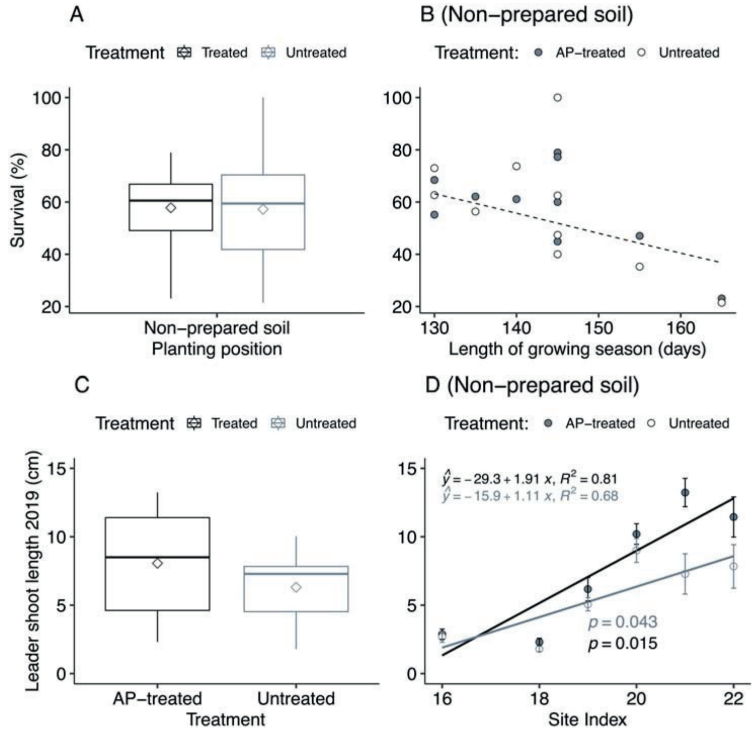

Figure 5. (A) Mean survival of seedlings planted in non-prepared soil with (black) or without (gray) arginine phosphate (AP) treatment across eleven study sites along a north-south gradient in northern Sweden. The diamonds indicate mean survival values for each position and treatment in (A) and mean 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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Disclosure statement

No potential conflict of interest was reported by the author(s).

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In Sweden, new forest stands have long been artificially regenerated using conifer seedlings. This approach may, however, need to be adapted to the changing climate. I investigated the effect of planting position, arginine fertilization and environmental variables on coated seeds and nursery-grown seedlings of Scots pine across 23 sites in Sweden. Planting nursery grown seedlings in mineral soil, with an amendment of arginine, appears to be the most valuable to enhance survival and growth of seedlings subjected to dry conditions.

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