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LIVESTOCK MANURE MULCHING AND BACTERIAL INOCULATION AS ALTERNATIVE STRATEGIES TO ENHANCE EARLY TREE ESTABLISHMENT ON SAND AND GRAVEL EXTRACTION SITES

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Land reclamation following the extraction of mineral resources (e.g., peat, sand, and gravel) is essential to restore the ecological functions and economic value of disturbed areas. Soils at sand and gravel extraction sites are typically impoverished — exhibiting low organic matter content, poor water retention, and unfavourable structure. To improve the fertility of such soils and support the successful establishment of forest stands and other vegetation, soil amendments are required. Among the most effective methods is the use of organic fertilizers, which improve soil structure and nutrient availability. This study was carried out in a reclaimed sand and gravel mining site where several tree species – silver birch, black alder, Norway spruce and Scots pine, were planted, and three types of organic livestock manure – swine, cattle, poultry, commonly used in agriculture, were applied as mulching materials. In addition, . Results indicated that cattle fertilizer enhanced the growth of birch, while poultry fertilizer promoted pine growth. However, the most pronounced effects were observed with swine additive, which positively influenced birch, alder, and pine. Spruce showed no response to any of the fertilization treatments. Across species, the addition of *Bacillus megaterium* had limited or species-specific effects, enhancing growth only in a few fertilizer combinations. Seedling type, moreover, did not account for a substantial proportion of the variation in height increment across the studied species.

Keywords: *land reclamation; sand and gravel extraction; soil amendment; organic fertilizers; afforestation*

INTRODUCTION

One of the most commonly extracted minerals in Latvia is sand and gravel, which are primarily used in construction, road building, and other industrial applications. In the territory of Latvia, approximately 3 million m³ of sand and a similar amount of sand–gravel is extracted annually (Latvian Environment, Geology and Meteorology Centre, 2024). Under EU legislation, including the Nature Restoration Law (Regulation (EU) 2024/1991), Member States are required to ensure that areas affected by mineral extraction are rehabilitated and ecologically restored promptly after the completion of extraction activities. One of the methods used to reclaim mineral soils is preparing them for subsequent use in forestry (Šebelíková et al., 2016).

Mineral extraction removes the topsoil entirely and causes severe degradation of soil properties, including loss of soil structure, increased erosion, excessive nutrient leaching, soil compaction, lowered pH levels, loss of organic matter, reduced nutrient availability for plants, and decreased microbial activity (Pagouni et al., 2024; Fauzan et al., 2022; Cummings et al., 2005, Prosser & Roseby, 1995). These changes collectively result in a decline in soil fertility. In sand quarries, a major factor negatively affecting the initial establishment of planted trees is the unstable moisture regime and excessive drought; however, by regulating soil conditions so that temperature fluctuations are minimized and moisture is retained, the potential for successful tree establishment can be significantly increased (Jacobs et al.,2005; Yang et al., 2017). Regulating soil moisture and supporting the initial establishment of planted trees can help improve other degraded soil properties, including soil structure, loss of soil organic carbon (SOC), soil biodiversity, and nutrient leaching (Li et al, 2024).

In mined areas, where unstable moisture regimes and topsoil dryness hinder tree establishment, mulching has been shown to effectively reduce soil evaporation, stabilize surface conditions, and limit erosion, thereby enhancing the prospects for vegetation recovery (Mulumba & Lal, 2008; Poesen & Lavee, 1991; Smets et al., 2008). Mulching materials can include a variety of sources, such as sewage sludge, cereal straw, shredded woody residues, compost (Rossi et al., 2024; Carabassa et al., 2018). Organic mulches not only promote sustainable soil productivity but also contribute to the recycling of waste from agricultural, forestry, and urban sectors (Peñaranda Barba et al., 2020). The use of poultry, cattle and swine manures for soil improvement in forest species has been investigated previously, and several studies report positive effects on seedling growth and nutrient availability (Friend et al., 2006; Heiskanen et al., 2022; Menes & Colombo, 1992). Using beneficial bacteria as a substitute for organic fertilizers can help overcome the slower nutrient dissolution often associated with them, compared with mineral fertilizers (Nabati et al, 2025). By accelerating nutrient transformation, these bacteria increase the pool of plant-available nutrients (Khan et al., 2024). Research shows that applying *Bacillus megaterium* to soil in agricultural systems can promote plant growth, help plants cope with pathogens, and increase the bioavailability of phosphorus and potassium for plant uptake (Xiaojia et al., 2013).

The effectiveness of mulching is strongly influenced by the type of material applied, prevailing climatic conditions, and the specific plant species targeted (Rossi et al., 2024). Most studies in sand quarries have been conducted in southern Europe, and outcomes may differ in northern regions, particularly under afforestation with native tree species (Peñaranda Barba et al., 2020). Nevertheless, the recognized challenges associated with introducing species that are effective in dry mineral soils—but may become invasive depending on the region—have stimulated interest in exploring native species as a safer alternative for mining area rehabilitation (Dinca et al., 2025)

Therefore, the aim of this study was to compare the effects of mulches derived from different organic manure sources on the growth of native tree species in a post-mining sand restoration area. We hypothesize that organic amendments will have a stronger positive effect on species that typically struggle to establish in sand quarries, such as *Alnus glutinosa*, while the effect will be less pronounced for species with a higher natural potential for afforestation without amendments, such as *Pinus sylvestris*.

RESEARCH METHODS

The research was conducted in a post-mining area in Latvia, located approximately 250 km northwest of Riga (57.33136, 25.95272). In the southern part of the sand-extraction reclamation site, a row-planting system was established, forming parallel lines. The spacing between rows was 4 m, and the spacing between tree plantings within each row was 1 m.

Native tree species – Silver birch *Betula pendula* Roth, Black Alder *Alnus glutinosa* (L.) Gaertn., Norway spruce *Picea abies* (L.) Karst. And Scots pine *Pinus sylvestris* L. were planted using both bare-root and containerized seedlings, depending on availability in the local market at the time of planting. In total 70 trees per species and seedling type were planted.

Mulch derived from three livestock manures (poultry, cattle, and swine)was applied. Each type of livestock manure-based fertilizer was inoculated with the bacterium *Bacillus megaterium*, a phosphate-solubilizing bacterium that improves P availability for plant uptake. In total, six different variations of mulch were used and a control group without application was created Table 1. Each species and planting material were established in separate rows, with different mulching materials applied at intervals of every ten trees.

Table 1. Experimental design

Tree species	Seedling type	Mulch material type (n=10)						
Silver birch	Bare root; improved root system	P	C	S	NA	P+B	C+B	S+B
	container	P	C	S	NA	P+B	C+B	S+B
Black alder	bare root; improved root system	P	C	S	NA	P+B	C+B	S+B
Norway spruce	bare root; improved root system	P	C	S	NA	P+B	C+B	S+B
	container	P	C	S	NA	P+B	C+B	S+B
Scots pine	bare root	P	C	S	NA	P+B	C+B	S+B
	container	P	C	S	NA	P+B	C+B	S+B

Abbreviations: P – Poultry manure organic mulching material; C – Cattle manure organic mulching material; S – Swine manure organic mulching material; NA- not applied, the addition of B (e.g., P+B, C+B, S+B) indicates that the respective organic mulching material produced from different manure or sludge composts is supplemented with the bacterium *Bacillus megaterium*. n = number of trees mulched

All substrate analyses were performed at the laboratory of the Latvian State Forest Research Institute "Silava." Soil pH was measured in a H_2O suspension. Concentrations of K, Ca, Mg, Zn, Fe, Mn, P, Al, Na, and S were determined after microwave-assisted digestion (CEM MARS 6 iWave, Matthews, USA) using an inductively coupled plasma optical emission spectrometer (ICP-OES; Thermo Fisher Scientific iCAP 7200 Duo, Waltham, USA) following ISO 11885:2009[16] Table 2. Total carbon (C) and nitrogen (N) contents were quantified by dry combustion (Elementar El Cube, Langenselbold, Germany) according to ISO 10694:2006 and ISO 13878:1998, respectively.

Planting was carried out in spring 2024, and mulching was applied immediately afterward around each seedling, at a rate of 0.7 kgDM per tree. The growth of the seedlings was assessed over two consecutive years by measuring their height with a precision of 1 cm using a measuring tape. During data preprocessing, records of damaged seedlings and those showing inconsistent height measurements were removed from the dataset.

Table 2. Chemical composition of fertilizers, g/kg dry mass.

Fertilizer, Manure	C	N	K	Ca	Mg	Zn	Fe	Mn	P	Al	Na	S	B	pH_{H2O}
Pig	333.0	10.8	9.909	58.962	8.196	0.455	4.403	0.817	5.004	4.773	1.085	8.523	0.050	7.53
Pig + bacteria	298.6	11.2	12.352	55.653	8.832	0.354	3.127	0.695	3.348	3.178	1.113	7.630	0.047	7.91
Cattle	295.8	11.3	12.136	47.774	8.030	0.305	2.446	0.643	3.037	2.673	1.073	6.838	0.044	7.80
Cattle + bacteria	321.1	11.1	8.913	52.212	8.109	0.363	3.315	0.688	4.086	3.468	1.012	7.209	0.044	7.77
Poultry	312.0	14.1	12.122	54.027	7.538	0.343	4.053	0.618	3.466	4.773	0.976	8.460	0.046	6.52
Poultry + bacteria	297.6	13.4	11.361	51.522	7.916	0.337	3.252	0.617	3.798	3.504	0.945	6.696	0.042	6.60

Planting was carried out in spring 2024, and mulching was applied immediately afterward around each seedling, at a rate of 0.7 kg DM per tree. The growth of the seedlings was assessed over two consecutive years by measuring their height with a precision of 1 cm using a measuring tape. During data preprocessing, records of damaged seedlings and those showing inconsistent height measurements were removed from the dataset..

To evaluate treatment effects on seedling performance, we fitted linear models of the form:

$$Increment_{ij} = \beta_0 + \beta_1 Seedling\ type_i + \beta_2 Fertiliser_j + \beta_3 (Seedling\ type_i \times Fertiliser_j) + \varepsilon_{ij},$$

where height increment (cm) was the response variable, fertiliser treatment (seven levels, including the control) and seedling type (bare-root or containerised) were fixed effects.

The residuals were normally distributed, we used Q-Q plots and histograms. For Scots pine and Norway spruce, two-year increments were analysed because the explanatory power was stronger comparing to annual increments. For silver birch and black alder, annual increments were used, because two year data gather during one observation time, was possible only for conifers. Black alder was represented only by improved bare-root seedlings; therefore, its model included only fertiliser treatment as predictor.

Each model was fitted both to the full dataset and to a subset excluding seedlings with top breakage. As parameter estimates and significance patterns were nearly identical between the two datasets, the full dataset was retained to better capture natural stand variability.

Pairwise contrasts between each fertiliser treatment and the control were obtained using estimated marginal means, and compact letter displays were used to identify treatments that did not differ significantly. A significance threshold of $\alpha = 0.05$ was applied, treatments sharing letters did not differ statistically in the figures. All analyses were performed in R using the packages emmeans, multcompView, dplyr, ggplot2, and broom (Graves et al., 2024; Lenth, 2024; Robinson et al., 2024; R Core Team, 2024; Wickham et al., 2023; Wickham, 2016).

RESEARCH RESULTS AND DISCUSSION

Seedling type

For birch, bare-root seedlings with improved root system tended to have slightly lower increment than container seedlings, but this difference was not statistically significant ($\beta = -3.16$, $p = 0.25$, $R^2 = 0.011$). In pine, no meaningful difference was observed between container and bare-root seedlings ($\beta = 0.06$, $p = 0.96$, $R^2 \approx 0$). Similarly, spruce bare-root seedlings with improved root system showed a small, non-significant increase in increment compared to container seedlings (i) ($\beta = 0.96$, $p = 0.19$, $R^2 = 0.016$). Overall, seedling type did not explain a substantial proportion of variation in height increment across the studied species.

Deciduous species

Cattle manure organic mulching material significantly increased growth of container-grown Silver birch seedlings (+14.8 cm, $p = 0.021$), whereas the combination of cattle manure with Bacillus megaterium (C+B) did not significantly differ from the control (+18.1 cm, $p = 0.476$). In contrast, some fertiliser–bacterium combinations reduced growth: for example, poultry manure with Bacillus megaterium (P+B) decreased increment compared to the control (–12.9 cm, $p = 0.049$). Other fertilisers, such as swine manure (S) or swine manure with Bacillus megaterium (S+B), showed variable effects, partly compensating for lower growth in bare-root seedlings. Overall, fertiliser type explained a substantial portion of variation in height increment among container-grown seedlings ($R^2 = 0.316$, $p = 0.003$) (Figure 1).

For container-grown black alder seedlings, fertiliser type had a modest effect on 2025 height increment, explaining a small proportion of variation ($R^2 = 0.124$, $p = 0.276$). Among the tested mulching materials, swine manure significantly increased growth compared to the control (+10.7 cm, $p = 0.047$), whereas other treatments, including cattle manure, poultry manure, and their combinations with *Bacillus megaterium*, showed no significant differences from the control. The estimated marginal means indicate that swine manure without bacterial supplementation promoted the highest growth (emmean = 29.9 cm), while poultry manure alone tended to reduce increment (emmean = 14.2 cm) (Figure 2).

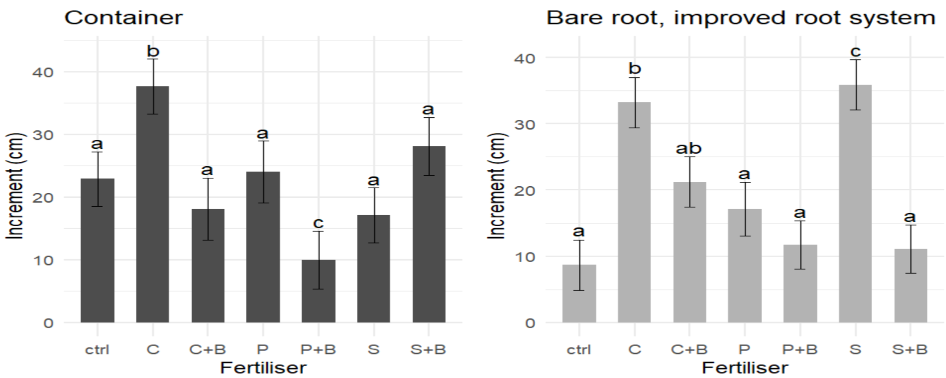

Figure 1. Silver birch (*Betula pendula*) current annual increment (cm) depending on different mulching material (P – Poultry manure organic mulching material; C – Cattle manure organic mulching material; S – Swine manure organic mulching material; NA- not applied, the addition of B (e.g., P+B, C+B, S+B) indicates that the respective organic mulching material is supplemented with the bacterium *Bacillus megaterium*) and seedling type.

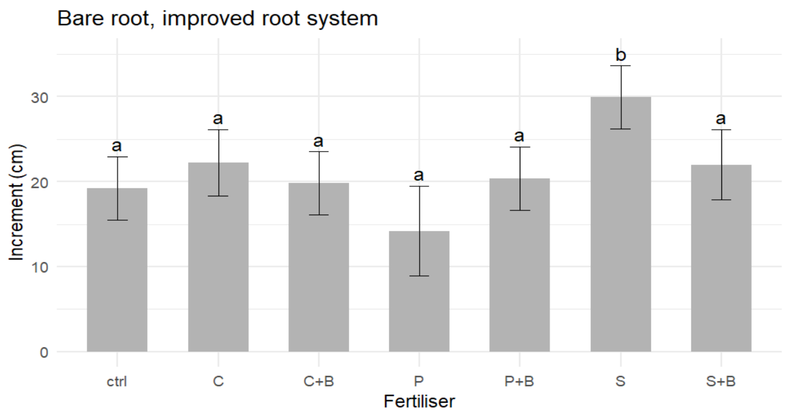

Figure 2. Black Alder (*Alnus glutinosa*) current annual increment (cm) depending on different mulching material type (P – Poultry manure organic mulching material; C – Cattle manure organic mulching material; S – Swine manure organic mulching material; NA- not applied, the addition of B (e.g., P+B, C+B, S+B) indicates that the respective organic mulching material is supplemented with the bacterium *Bacillus megaterium*).

Conifer species

Among container-grown spruce seedlings, most fertiliser treatments had limited effects on 2025 height increment ($R^2 = 0.16$, $p = 0.161$). Swine manure supplemented with *Bacillus megaterium* (S+B) tended to increase growth compared to the control (+3.41 cm, $p = 0.062$), while other fertilisers, with or without bacterial addition, showed no significant effects. For bare-root seedlings with an improved root system, no fertiliser treatment differed significantly from the control ($R^2 = 0.098$, $p = 0.540$), although some combinations, such as cattle manure with *Bacillus megaterium* (C+B) and poultry manure alone (P), tended to reduce growth, while poultry manure with bacteria (P+B) and swine manure treatments showed small positive effects. Overall, fertiliser type had a minor influence on spruce height increment, and only S+B in container seedlings showed a near-significant positive effect (Figure 3).

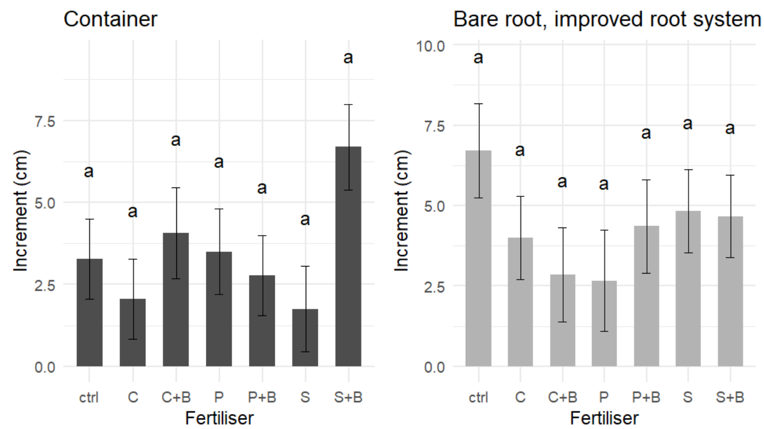

Figure 3. Norway spruce (*Picea abies*) two-year increment (cm) depending on different mulching material (P – Poultry manure organic mulching material; C – Cattle manure organic mulching material; S – Swine manure organic mulching material; NA- not applied, the addition of B (e.g., P+B, C+B, S+B) indicates that the respective organic mulching material is supplemented with the bacterium Bacillus megaterium) and seedling type.

Several mulching materials positively affected Scots pine height increment, but the magnitude of the effect differed between container-grown and bare-root seedlings. In container seedlings, swine manure (S) significantly increased growth (+6.79 cm, p = 0.026), while other treatments, including cattle manure with *Bacillus megaterium* (C+B) and poultry manure treatments, showed non-significant trends (emmean range: 4.1–16.7 cm). For bare-root seedlings with improved root systems, multiple fertiliser treatments significantly enhanced growth: C+B (+6.98 cm, p = 0.007), P+B (+10.34 cm, p < 0.001), S (+8.81 cm, p = 0.001), and S+B (+8.44 cm, p = 0.005), while other treatments showed smaller or non-significant effects. Overall, these results indicate that the response of Scots pine to mulching depends on both fertiliser composition and seedling type, with bare-root seedlings generally showing larger positive effects from fertiliser–bacterium combinations (Figure 4).

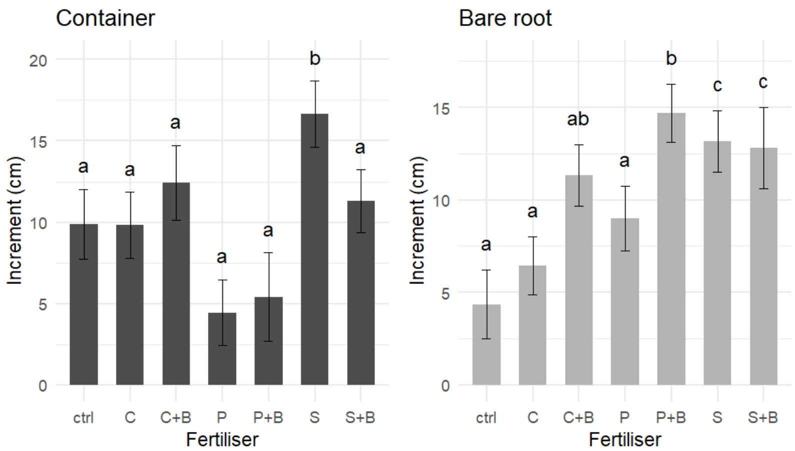

Figure 4. Scots pine (*Pinus sylvestris*) two-year increment (cm) depending on different mulching material (P – Poultry manure organic mulching material; C – Cattle manure organic mulching material; S – Swine manure organic mulching material; NA- not applied, the addition of B (e.g., P+B, C+B, S+B) indicates that the respective organic mulching material is supplemented with the bacterium Bacillus megaterium) and seedling type

Overall, mulching effects varied across species, with the strongest and most consistent positive responses observed in pine. Silver birch responded positively to cattle manure organic mulching in container seedlings, while bare-root seedlings showed slightly lower increments, with some fertiliser–bacterium combinations partly compensating for this difference. In black alder, swine manure increased growth in container seedlings, whereas other treatments, including cattle and poultry manure with or without *Bacillus megaterium*, had limited or non-significant effects. Spruce seedlings showed minor responses overall, with only swine manure + *Bacillus megaterium* in container seedlings exhibiting a near-significant positive effect; bare-root seedlings were largely unresponsive. Scots pine displayed the clearest gains, with multiple fertiliser treatments enhancing growth, particularly in bare-root seedlings (C+B, P+B, S, S+B), while container seedlings showed smaller or non-significant responses. These results indicate that both mulching type and seedling material influence growth, with conifers generally more responsive to fertiliser treatments than broadleaved species. Previous studies on mineral soils show that nitrogen is the primary nutrient driving conifer growth responses and that its application alone can produce substantial effects (Nilsen, 2001; Nohrstedt, 2001). In contrast, black alder, being a nitrogen-fixing species, may show little or no response to nitrogen treatment (Sroka et al., 2018). Pine, which exhibited higher nutrient deficiencies based on biomass analyses compared with alder, is therefore likely to respond more strongly to mulching (Kuznetsova et al., 2010).Poultry fertiliser produced the weakest effects compared with swine and cattle fertilisers, enhancing growth only in pine when combined with *B. megaterium*. Previous studies reported improved pine growth on forest soils following poultry fertilisation (Friend et al., 2006). However, other research has also indicated risks of over-fertilisation with poultry manure, suggesting that the application rate used in this study was likely excessive (Shepherd & Bhogal, 1998). Livestock manure has previously been shown to enhance birch and pine growth (Heiskanen et al., 2022). In this study, manure compost also produced positive growth responses, although not in all cases. These differences may be explained by the use of composted manure, whereas earlier results were obtained with digestate. Anaerobic digestion stabilizes the manure substrate, reducing its biological activity and variability, which may partly account for the observed discrepancies (European Biogas Association, 2024).

CONCLUSIONS

Organic mulching enhanced seedling growth in post-mining sand areas, with the strongest and most consistent effects observed in Scots pine and, to a lesser extent, Norway spruce. Several treatments, especially swine manure, cattle manure + *Bacillus megaterium*, and poultry manure + *B. megaterium*, significantly increased pine growth, while responses in deciduous species were weaker—silver birch benefited from cattle manure mulch, and black alder showed limited gains. Contrary to our initial hypothesis, black alder did not respond more strongly to organic amendments than species with higher natural afforestation potential. The addition of *B. megaterium* sometimes enhanced growth but also caused negative interactions in some seedlings, indicating its effects are species- and seedling type-dependent. Overall, mulching is an effective restoration tool for conifers, with cattle- and swine-manure based mulches being the most promising options.

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