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# **Effects of microbiome on conifer health and resistance to biotic and abiotic stress: A systematic review**

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Master of Science in Ecology

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Ås, June 2023

Marrian Tendai Rwizi

## ABSTRACT

The dynamics of plant-microbiome interactions under different stress conditions are important to understand in order to elucidate the mechanisms underlying forest decline and tree mortality. This study aims to evaluate the importance of ectomycorrhizal fungi in alleviating stress and enhancing defense in *Pinus* and *Picea* species. A meta-analysis was performed on primary research articles that assessed effects of ectomycorrhizal fungal on growth and resistance of these tree species. The stress factors studied were drought, pathogen infection, herbivory and pollutant stress. A PRISMA systematic review guideline was used in screening the articles from the two databases Web of Science and SCOPUS. The search produced a total of 1 806 articles, which were further screened by excluding review articles, book chapters, conference papers and other papers that did not include *Pinus* or *Picea* species and ectomycorrhizal fungi. A total of 118 articles were assessed and the statistical analysis was conducted as per factor (growth, survival, biotic and abiotic stress). Most of the published articles were on *Pinus* and a few on *Picea*. Overall, ectomycorrhizal fungi were found to enhance growth but had no effect on survival, though the results are more confined to *Pinus* species. Ectomycorrhizal fungi were not effective in alleviating abiotic and biotic stress. There was high heterogeneity among papers and publication bias in the analysis. As a result my analysis did not provide a concrete conclusion in endorsing ectomycorrhizal fungi as promoting conifer growth, survival and resistance to biotic and abiotic stress. Further research is needed on conifer- soil feedbacks in relation to ectomycorrhizal fungi. Moreover, there is need for conducting more experiments in the field to enable the life strategies of ectomycorrhizal fungi to be brought into sharper focus. This is important because conifer species are of high economic value through producing high quality timber and plays a crucial role in mitigating climate change.

Key words: Ectomycorrhizal fungi, *Pinus*, *Picea*, heterogeneity, publication bias, growth, survival, biotic and abiotic.

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## **LIST OF ABBREVIATIONS**

ECM- Ectomycorrhizal fungi,

AM- Arbuscular mycorrhizal fungi

REML- Random effects model

## **1. INTRODUCTION**

Plants and their associated microbes have been interacting since the colonization of land by ancestral plants 450 million years ago. This assemblage of a plant host and its microbiome is called the holobiont (Baedke et al, 2020). Plant- microbiome relationships can be commensal or mutualistic, i.e. benefiting both partners (Bacon & White, 2016) (Van der Ent et al., 2009).

The microbiome includes organisms found below ground (in the rhizosphere) or above ground (phyllosphere). The rhizosphere is inhabited by a variety of microorganisms including bacteria, fungi, oomycetes, nematodes, protozoa and archaea. Mycorrhiza fungi are the most abundant members of the rhizosphere community with an estimated 80% plant association and have been found in over 200 000 plant species (Dastogeer et al., 2020). They play a major role in terrestrial ecosystems and are major drivers of carbon and nutrient cycles (van der Heijden et al., 2015). In the phyllosphere, microbes are very much affected by changes in temperature and moisture which may affect the plant. In addition to the rhizosphere and phyllosphere there is the endosphere. To colonize this internal compartment, microbiota must penetrate a plant's external boundary and overcome or hide from plant defenses (Bulgarelli et al., 2012). Arbuscular mycorrhizal fungi (AM) and other endophytic fungi are dominant colonizers of the endosphere (Dastogeer et al., 2020).

For trees to flourish they need Nitrogen, Phosphorus and water from soil, but the levels of these nutrients in soil are often too low to sustain tree growth and so the trees rely on ectomycorrhizal symbiosis to help them survive (Martin et al., 2016). Ectomycorrhizal fungi (ECM) can establish mutualistic symbiosis with a wide range of woody plants, including conifers and other gymnosperms, particularly in sites where nutrients are low and limiting (Read et al., 2004). They play a major role in temperal and boreal forests by providing soil nutrients and water in exchange of carbon, helping their host trees tolerating harsh environmental conditions (Policelli et al., 2020).





**Figure 1:** Mycorrhization of white bark seedlings with ectomycorrhizal fungi

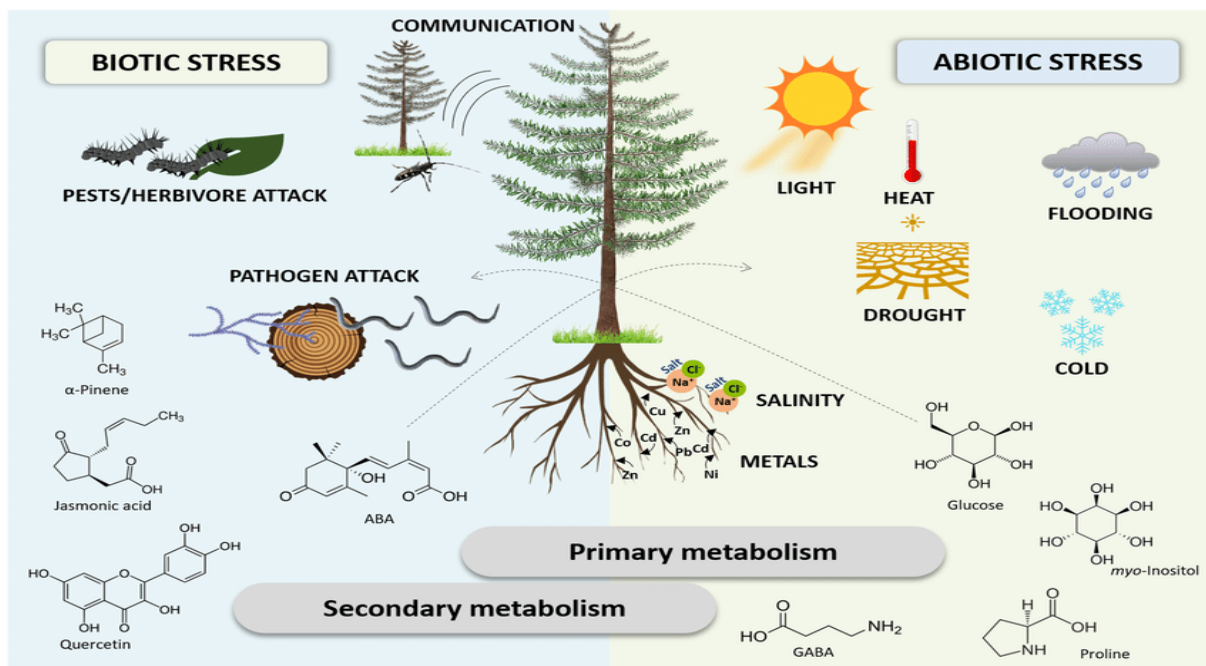
Successful mycorrhizal colonization of white-bark (*Pinus albicaulis*) pine seedlings with a native *Suilloid* species in a greenhouse. White areas are ectomycorrhizal fungi which have colonized the roots (Lonergan, 2013).

Due to their large hyphal network in the soil (Figure1), ECM enhance the absorbing root surface of the plant host for mineral nutrients. In exchange, the plant host provides carbon to the mycorrhizal fungi(Smith, 2008). Additionally, ECM can be host- specific in response to the type of stress, affecting nutrient uptake, and leading to reduced plant growth (Taniguchi T, 2017).

Fluctuating environmental conditions can cause a plant to be attacked by pathogens and subsequent herbivore and or insect attack. In response to these attacks, plants have preformed and inducible defense mechanisms (Iqbal Zahra et al, 2021). Conifers such as Norway spruce can live for more than 500 to 600 years (Castagneri et al., 2013). One factor contributing to the longevity of conifers is their defensive strategies, the mechanisms they evolved against attack from insects and pathogens (Franceschi et al., 2005). In constitutive defense mechanisms, preformed barriers such as the cell wall, epidermal cuticle and the bark, protects the plant from attack and invasion. Preformed defenses can also be chemical defenses in which the tree produces anti-feedants, toxins, proteins and enzymes that are distributed in the

bark and wood (Franceschi et al., 2005). Some proteins are specifically targeting certain microbes as shown in the antifungal activity of defensins (Thomma et al., 2002).

Inducible defense mechanisms increase the efficiency of the plant defense system and involve e.g. chemical defenses such as the release of secondary metabolites (phenolics, terpenoids and alkaloids) that can defend the tree against a wide range of pests or herbivore attack (Franceschi et al., 2005) and abiotic stresses such as drought, pollutants and salinity (Rodrigues et al., 2021). Plants respond to these stresses through different ways, for example they can escape from drought by adjusting their life cycle, decreasing the osmotic potential in their cells and upregulating antioxidant defenses. Molecular mechanisms include synthesis of stress proteins, and signalling stress detection (Athar et al., 2022). Several studies have documented the importance of mycorrhizal fungi in counteracting biotic stress in plants, including effects on pathogenic fungi such as *Fusarium*, *Rhizoctona*, *Verticillium*, *Thielavopsis*, *Aphanomyces*, *Phytophthora* and *Pythium* (Whipps, 2004), as well as nematodes from the genera *Heterodera*, *Meloidogyne*, *Pratylenchus* and *Radopholus* (Harrier & Watson, 2004).



**Figure 2:** Chemical defense mechanisms of trees

Chemical defense mechanisms of trees by realising secondary metabolites in response to abiotic and biotic stress (Rodrigues et al., 2021).

During the past decades, individual studies have generated sufficient evidence on responses of plants to ectomycorrhizal associations that some conclusions can now be made about the nature of these associations. However, the published results are inconsistent to some extent. Some studies recorded positive effects of ectomycorrhizal fungi when administered to conifers (Kennedy et al., 2007) and others reported negative microbial responses (Herol et al., 2022). However, there has been not enough quantitative synthesis that allows us to determine the effect of ectomycorrhizal fungi on conifer (*Pinus* or *Picea*) health, survival and defense. More systematic knowledge can be achieved through a meta-analysis, which is a method to summarise results of multiple independent studies, identifying conflicting published studies (Koricheva & Gurevitch, 2013), highlighting research gaps in data, and identifying common methodological problems (Lortie & Callaway, 2006).

### **1.1 AIM**

Hitherto, most information on beneficial effects of mycorrhizal plant-fungal interactions has been gathered on arbuscular mycorrhizal fungi (AM) known to form symbiotic associations with many crop plants. Less attention has been given to ectomycorrhizal fungi (ECM) that are specific for symbioses with woody plants. There is thus a need for understanding the complex relationship between woody plants and their microbiota (particularly ectomycorrhizal fungi) in enhancing defense to biotic and abiotic stress. This need is especially pressing for forest trees such as the conifers which are crucial for future forest productivity and forest restoration.

### **1.2 OBJECTIVES**

The main objective of this thesis is to evaluate to what extent ectomycorrhizal fungi in enhance growth and resistance to biotic and abiotic stress of conifers in the genera *Pinus* and *Picea*. I do this by conducting a meta-analysis of published primary research articles that have manipulated ectomycorrhizal fungi and other variables that has assessed its effect on growth and resistance to biotic and abiotic stress.

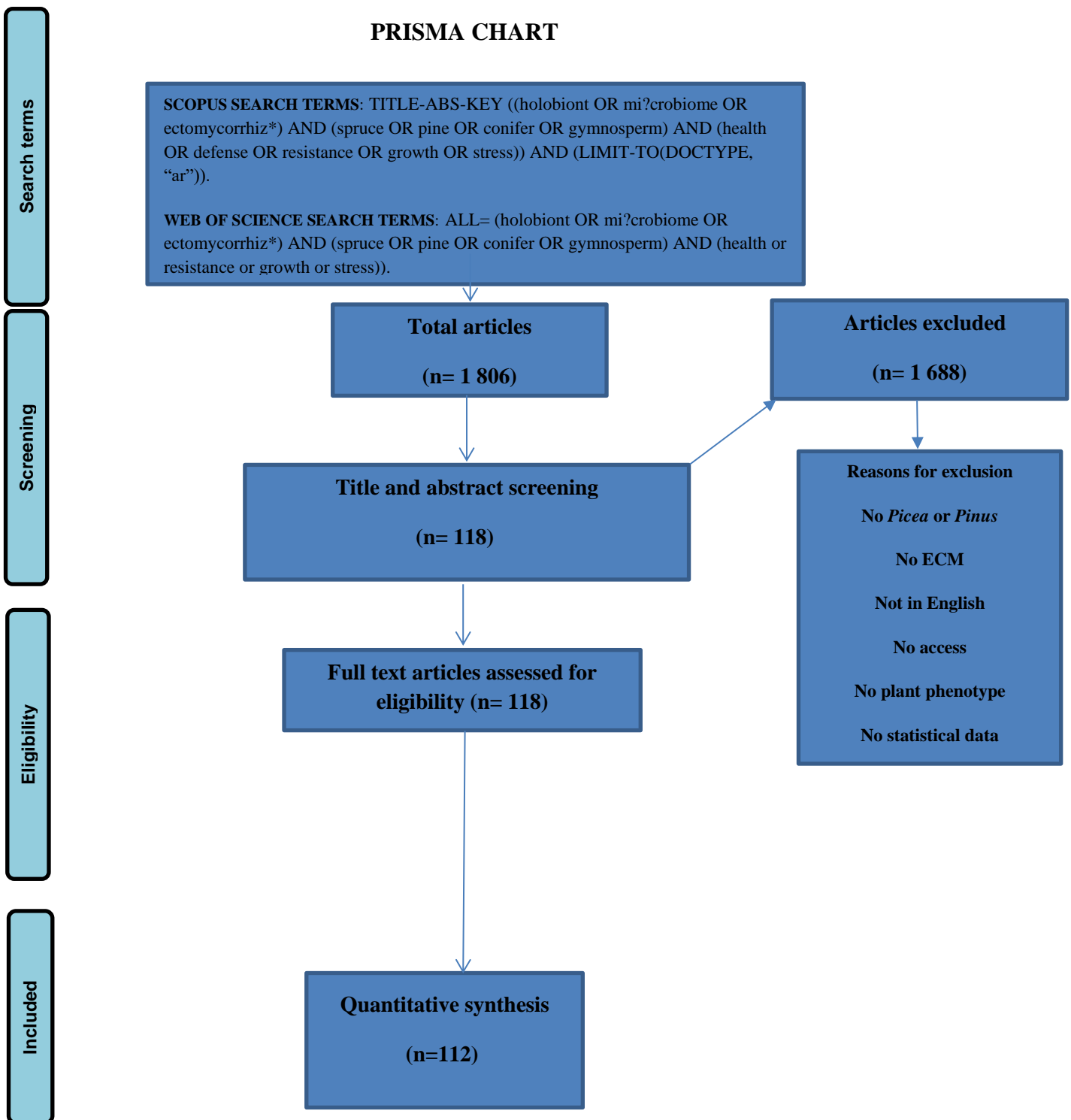
### **1.3 RESEARCH QUESTIONS**

- Is there is scientific evidence that ectomycorrhizal fungi enhance plant growth, survival and defense to biotic and abiotic stress?
- Does the experiment location or growth condition affect the outcome of results?

## 2.0 METHOD

### 2.1 Literature search

The meta-analysis was performed in concordance with the PRISMA systematic review guidelines (BMJ, 2021) (Figure 3). A literature search was performed using the databases; Web of Science (<https://www.webofscience.com/>) and SCOPUS (<https://www.scopus.com/>). Terms used to search Web of Science were: ALL= (holobiont OR mi?crobiome OR ectomycorrhiz\*) AND (spruce OR pine OR conifer OR gymnosperm) AND (health or resistance or growth or stress)). The search terms used in SCOPUS were: TITLE-ABS-KEY ((holobiont OR mi?crobiome OR ectomycorrhiz\*) AND (spruce OR pine OR conifer OR gymnosperm) AND (health OR defense OR resistance OR growth OR stress)) AND (LIMIT-TO (DOCTYPE, "ar")). Initially, the two searches identified 1 806. Abstracts and meta data for all articles were imported into Rayyan (<https://www.rayyan.ai/>), a web-tool to help researchers working on systematic reviews, so they could be individually reviewed. Each article was reviewed by two independent reviewers (me and one of my supervisors). Articles that did not deal with *Picea* or *Pinus*, ECM and/or the effects of ECM on conifer growth, survival, abiotic stress or biotic stress were excluded. Most articles that were published in the 1980s and 1990s were not accessible online, thus they were excluded for the meta-analysis. Additionally, articles for which there was no online access, that were not written in English, or were not primary research articles were excluded. In the end, 118 articles were found suitable for meta-analysis. Of these 118 studies, only 112 articles had enough statistical data or other key information required to perform the meta-analysis (Figure 3).



**Figure 3:** Prisma chart describing the screening process.

PRISMA chart describing the search protocol and screening process used to identify and select published research articles for this systematic review.

## 2.2 Data extraction

I read each of the 118 articles included in the meta-analysis and harvested data that could be used to calculate effect sizes (See Appendix E). The effect of ECM on tree growth, survival, biotic, and abiotic was investigated. The data parameters used in the study include seedling height, stem diameter, shoot length, and stem length, root length, survival and mortality (See Appendix B, C, D and A). For articles that did not provide data in the text, tables, or supplementary data files, Image J image analysis software (<https://imagej.net/ij/>) was used to derive data from figures. Mean and standard deviation or standard error values from data graphs were determined by measuring pixels and scaling these to the y-axis units. I also recorded additional factors that could influence the effect sizes. These possibly moderating factors were tree genus, tree age, ECM genus, soil type, and growth condition and stress type. Soil types were grouped into four categories: field, composite, sandy and media. Soil from the forest or field was categorised as field. Nursery soils, i.e. various mixtures of soil substrates such as vermiculite and peat, were categorised as composite soil. Studies using sand as the main substrate were classified as sandy soil. Liquid substrates and agars were classified as media. Studies that were conducted under controlled environments were coded as growth facility (greenhouse, glass house, lab, and nursery) and those which were carried out in the field were coded as field. Stress types were coded as heavy metals, pathogen infection, drought and insect attack.

## 2.3 Effect size standardisation and normalisation

Effect sizes were calculated following the method presented in the Hard-boiled synthesis protocols (Lajeunesse, 2016). Data extracted from the literature was normalised and calculated using the following formulae and steps:

1. The difference of  $\delta$  between the means ( $X$ ) of ECM treatment (T) and control was calculated:

$$\delta = X_T - X_C \quad (1)$$

( $X_T$  and  $X_C$  are treatment and control means)

2. The homogenised variance ( $\sigma$ ) of the means of treatment and control was calculated:

$$\sigma = \sqrt{((N_T - 1) SD_T^2 + (N_C - 1) SD_C^2) / (N_T + N_C - 2)} \quad (2)$$

( $N_T$  and  $N_C$  are treatment and control sample size)

3. I then standardised delta relative to sigma :-

$$\delta/\sigma \text{ (delta/sigma)} \quad (3)$$

4. and estimated data variance of effect size.

$$\text{Var}(\delta)=1/N_T+1/N_C+ \delta^2/2(N_T+ N_C) \quad (4)$$

5. performed bias correction:

$$\mathbf{J} = 1-(3/4 \times (4 \times (N_T+ N_C-2)-1)) \quad (5)$$

**(J is the bias correction)**

6. Calculated delta ( $\delta$ )

$$\delta = X_T-X_C/ \sigma \times \mathbf{J} \quad (6)$$

7. Calculated variance of the delta

$$\delta = \sqrt{\text{Var } \delta *j^2} \quad (7)$$

8. And finally calculated effect size from Var  $\delta$ .

$$\text{Var}(\delta) = 1/N_T + 1/N_C + \delta^2/2(N_T +N_C) \times j^2 \quad (8)$$

EFFECT SIZE

$$(\delta) = X_T-X_C/ \sigma$$

Data that were presented as proportions or percentages were converted to log response ratios (log RR) using the following formulae and steps:

1. Risk ratio (RR) was first calculated:

$$\text{RR} = P_{\text{treatment}}/P_{\text{control}} \quad (9)$$

**(P is the proportion)**

2. Response ratios were then transformed to log RR using the formulae:

$$\text{RR} = \text{LN}(\text{RR}) \quad (10)$$

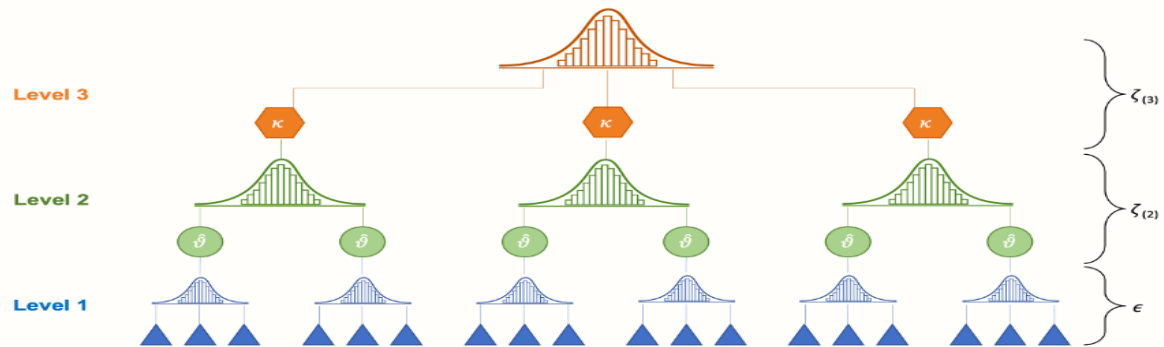
3. and standard errors for log risk ratios were calculated.

$$\text{SE log RR} = (1/K_T+1/K_C - 1/N_T+1/N_C) \quad (11)$$

( $N_T$  and  $N_C$  are treatment and control sample size, and  $K$  is the number of events e.g., dead plants)

## 2.4 Data modelling and determination of pooled effect size

The overall effect of ECM fungi on their host was estimated using the METAFOR package by Viechtbauer (2010) in R (R version 4.2.3), by fitting a three level meta-analysis random effects model (Harrer et al., 2021). Level 1( $\epsilon$ ) were individual data points (effects) within studies. In level 2  $\zeta(2)$ , the individual data points that were clustered (K) by study. In level 3  $\zeta(3)$ , the effect sizes from all studies were pooled into an overall estimated effect size (average).



**Figure 4:** Three level meta-analysis model

Diagram of a three level meta-analysis model used in this study (Harrer et al., 2021). Level 1 shows data points of individual studies and their effect sizes. Level 2 shows effect sizes of subgroup studies aggregated together where each study contributes only one effect size. Level 3 is the overall true pooled effect size from subgroups in level

The model equation used was:

$$\hat{\theta}_{ij} = \mu + \zeta(2)_{ij} + \zeta(3)_j + \epsilon_{ij}$$

Where  $\hat{\theta}_{ij}$  is an estimate of the true effect size,  $\mu$  is the true effect,  $i$  are some effect size nested in cluster which can be a subgroup  $j$ ,  $\zeta(2)_{ij}$  and  $\zeta(3)_j$  represent heterogeneity within clusters. The function used to run the model in R was:

```
full. Model <- rma.mv (yi = effect size,
  V = var. d,
  slab = author,
  data = x,
  random = ~ 1 | author/es.id,
  test = "t",
  method = "REML")
```



Where  $y_i$  is the calculated individual effect size and  $V$  is the calculated variance delta. The author is representing individual studies (level 2) and  $es.id$  is representing individual effects (level 1).

The estimated pooled effect size was also transformed to a normal correlation to facilitate, easy interpretation of results using the function: `convert_z2r ()`.

Additional factors (moderators) such as soil type, growth condition, tree age, stress type and tree genus were assessed for their overall effects on the pooled effect size using the equation:

$$\hat{\theta}_{ij} = \theta + \beta x_i + \zeta(2)_{ij} + \zeta(3)_j + \epsilon_{ij}$$

Where  $\theta$  is the intercept and  $\beta$  the regression weight of a predictor variable for instance soil type.

These factors were specified in `rma.mv ()`, using the `mods` argument. A three- level moderator model was used using the above equation:

```
mod. model <- rma.mv(yi = effect size, V = var. d,
  slab = author,
  data = data x,
  random = ~ 1 | author/es.id,
  test = "t",
  method = "REML",
  mods = ~ moderator x)
```

Where  $y_i$  is the effect size and  $V$  is the variance delta.

## 2.5 Distribution of variance across levels

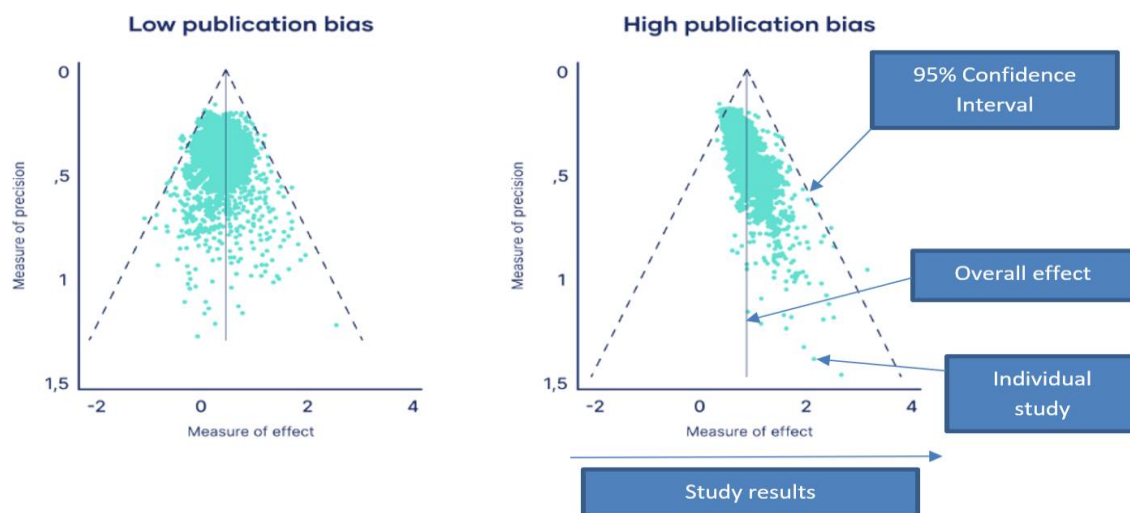
Higgin's and Thompson's  $I^2$  statistic was used to quantify heterogeneity between study levels based on Cochran's  $Q$ . This is defined as the percentage of variability in the effect sizes in which the sampling error is not the cause. The formular for  $I^2$  used is:

$$I^2 = \frac{Q - (K - 1)}{Q}$$

Where  $K$  is the total number of studies, and  $Q$  is the Cochran's  $Q$ , which is the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method (Higgins & Thompson, 2002).

## 2.5 Bias detection

The METAFOR package by Viechtbauer (2010) in R (R version 4.2.3) was used to create funnel plots to evaluate bias in the study and to determine the validity of the results, using the function: `funnel()`.



**Figure 5:** Diagram of a funnel plot

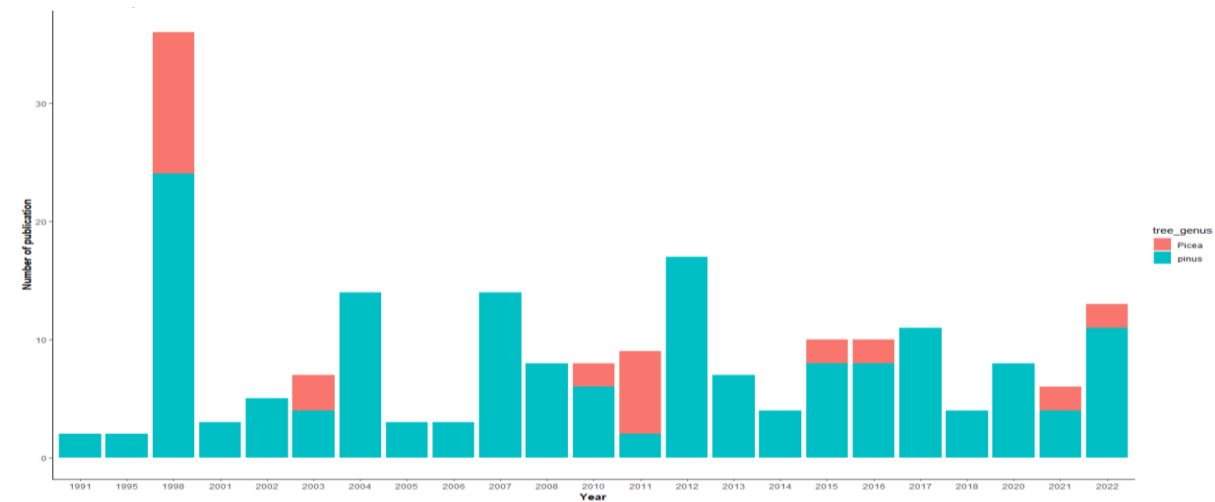
An example of a funnel plot modified from (<https://www.scribbr.com/frequently-asked-questions/funnel-plot-publication-bias>) Funnel plot showing publication bias of growth studies. Each dot represents a study (measuring effect of ectomycorrhizal fungi). The y-axis represents the study precision (standard error) and the x-axis shows the study outcome (effect size). The outer dashed lines show 95% Confidence Interval limits. The average effect size is shown by the dashed line in the middle. Larger and most powerful studies are placed towards the top. In the absence of a bias the scatter will resemble a symmetrical plot.

### 3.0 RESULTS

Out of 1806 searched articles, 118 articles were found suitable for meta-analysis but only 112 articles had enough data and other key information required to perform the meta-analysis.

#### 3.1 Effects of ECM on conifer tree growth

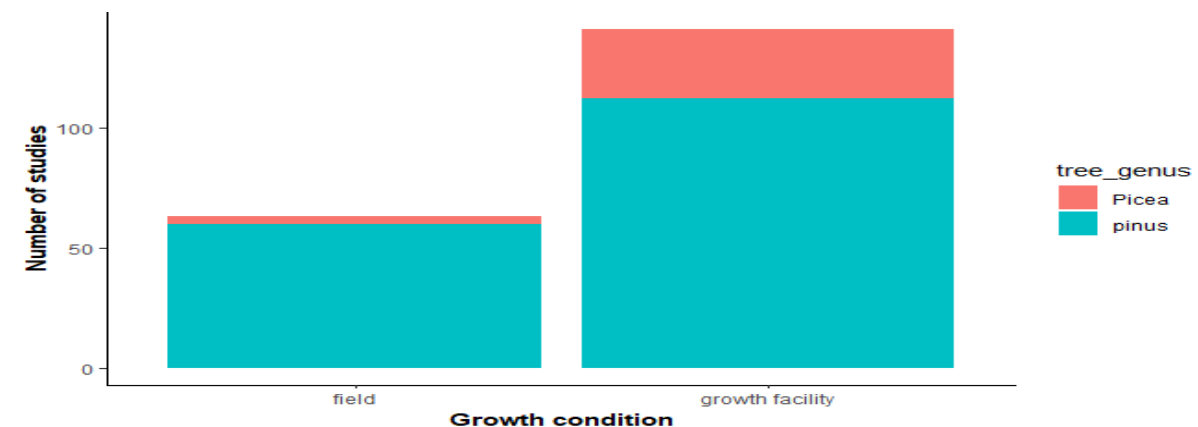
There are many articles on *Pinus* species compared to *Picea* species. The years with the most articles are 1998, 2004, 2005, 2007 and 2012, and all these years were dominated by *Pinus* species (Figure 6).



**Figure 6:** Number of publications on conifer tree growth

Publication year of articles included in the meta-analysis of ECM effect on *Pinus* and *Picea* growth.

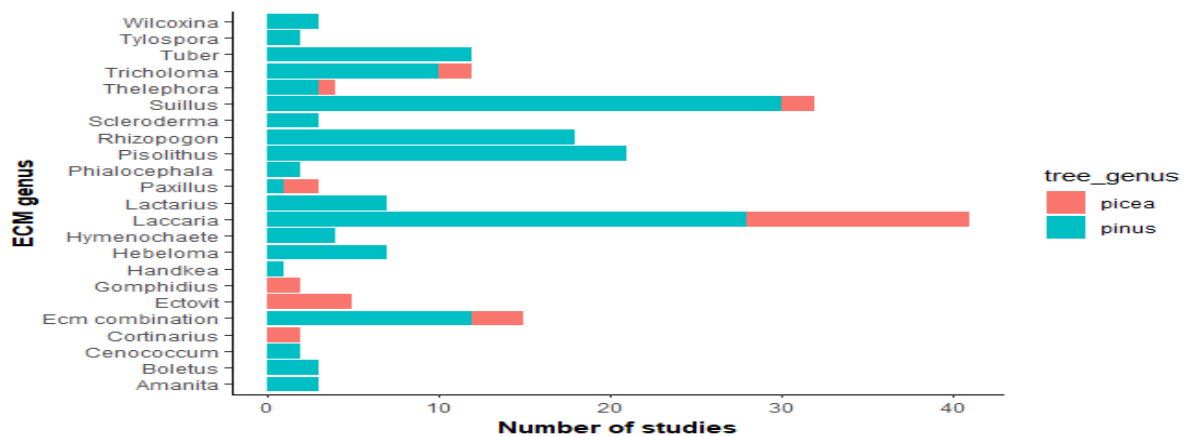
Growth studies were mostly conducted in a growth facility, and much of the investigation was conducted on *Pinus* (Figure 7). There was a total of 32 studies conducted on *Picea* growth and 172 studies conducted on *Pinus* growth.



**Figure 7:** Growth conditions under which growth studies in the meta-analysis were conducted.

Growth condition under which studies included in meta-analysis of ECM effect on *Pinus* and *Picea* growth were conducted. Studies conducted under controlled environments, greenhouse, glasshouse, lab, or nursery, were allocated to “growth facility” and those which were carried out in the field were allocated to “field”.

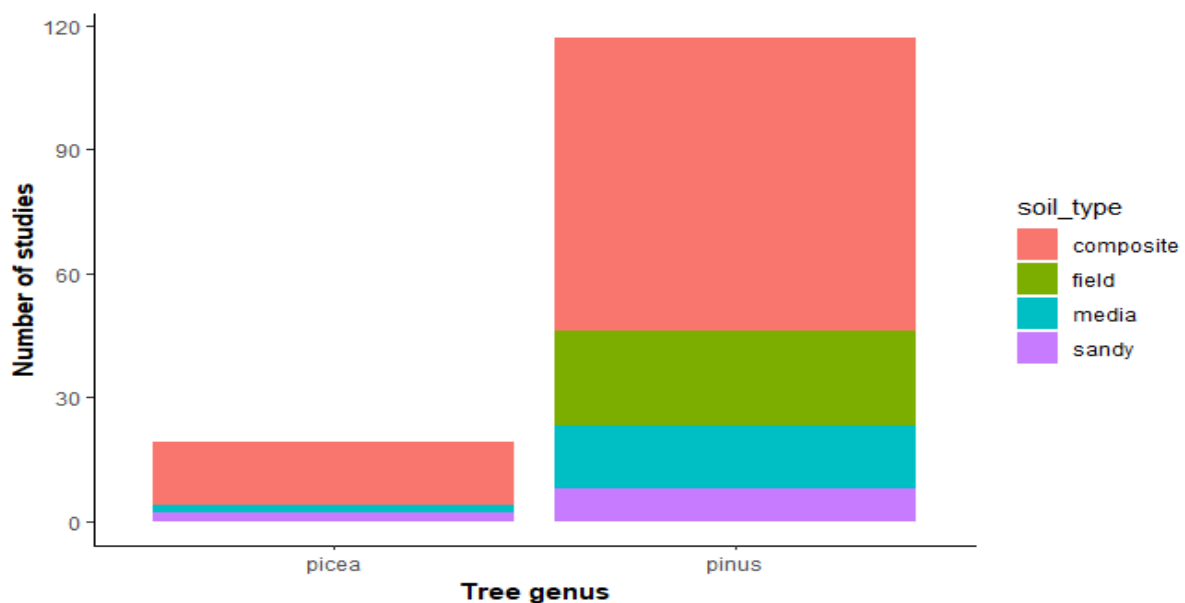
The most common ECM genera used were *Suillus*, *Rhizopogon*, *Laccaria* and *Pisolithus*, though ECM was mostly tested on *Pinus* species (Figure 8).



**Figure 8:** ECM genera which were used for growth studies in the meta-analysis.

ECM genera used in studies included in meta-analysis of ECM effect on *Pinus* and *Picea* growth were conducted. ECM denotes ectomycorrhizal fungi where more than one ECM genus was used. Ectovit is a mycorrhizal blend manufactured for commercial use.

The composite soil type was the most used on both *Picea* and *Pinus* species. Sandy was the least used soil type (Figure 8).

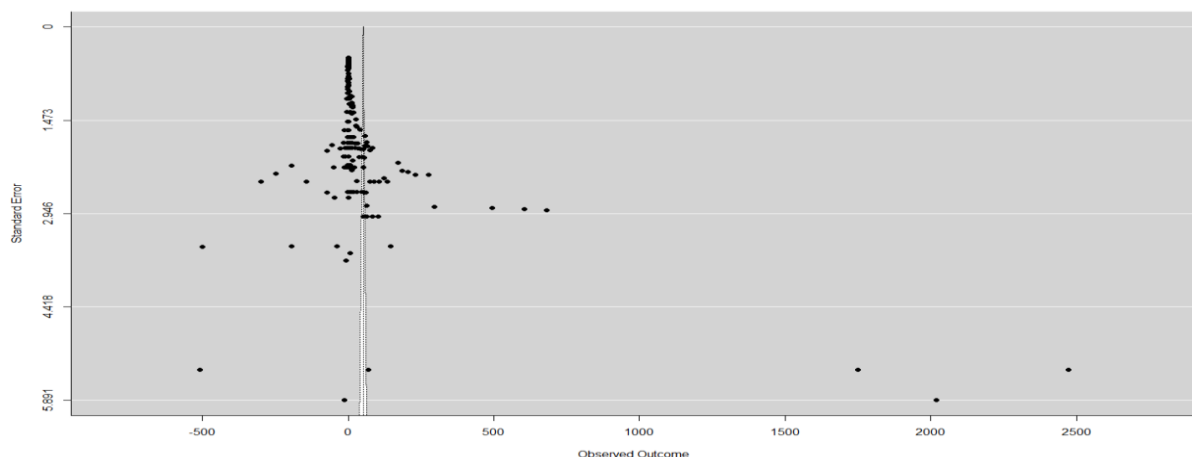


**Figure 8:** Soil type used on conifer tree growth studies.

Soil from the forest or field was categorised as field. Nursery soils, i.e., various mixtures of soil substrates such as vermiculite and peat, were categorised as composite soil. Studies using sand as the main substrate were classified as sandy soil. Liquid substrates and agars were classified as media.

The overall pooled estimated effect size based on the three -level meta -analysis model was 51 (95% CI: 1.73- 100;  $p=0.04$ ). The large estimated pooled effect size indicates a very strong relationship between ectomycorrhizal fungi and tree growth. There was a high level of heterogeneity between individual studies and within studies.  $I^2_{\text{level } 3}$ (overall effect size) was 23.27% and  $I^2_{\text{level } 2}$  (nested effect sizes from individual studies) was 76.73(Figure 10).

There is an indication of publication bias on tree growth factor because the plot is not symmetrical. Excessive number of studies do not fall within 95% confidence interval limit (Figure 9) and thus are statistically significant. Therefore, publications are biased although their studies are significant.



**Figure 9:** Funnel plot of publication bias of growth studies

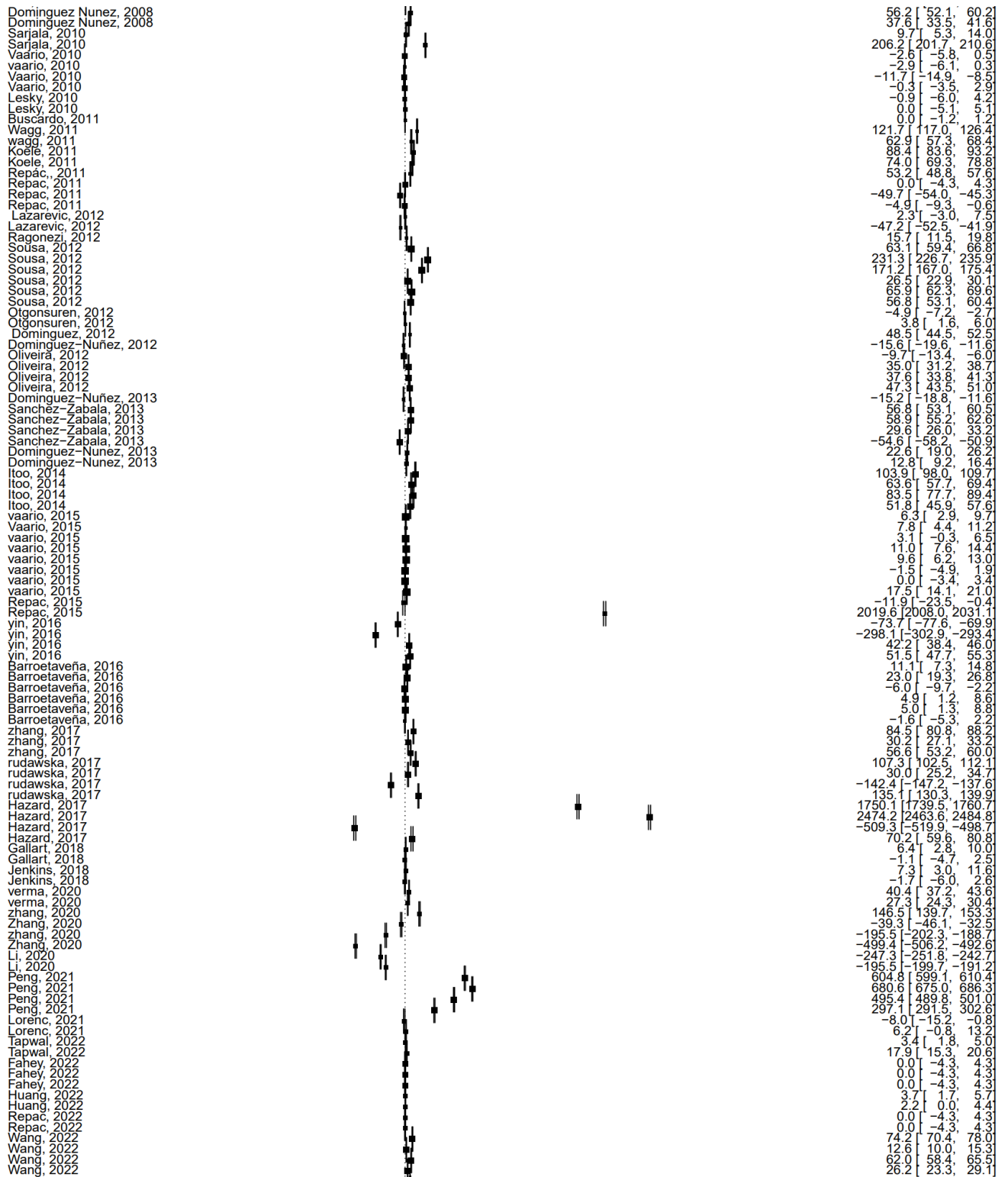
Funnel plot showing publication bias of growth studies. Each dot represents a study (measuring effect of ectomycorrhizal fungi). The y-axis represents the study precision (standard error) and the x-axis shows the study outcome (effect size). The outer dashed lines show the 95% Confidence Interval limit. The average effect size is shown by the dashed line in the middle. Larger and most powerful studies are placed towards the top. In the absence of a bias the scatter will resemble a symmetrical inverted pollen.

The pooled estimated effects sizes of the moderators were huge indicating a positive moderating effect on the relationship between ectomycorrhizal fungi and conifer tree growth with no significance ( $P > 0.05$ ), except for soil type which had a p value of 0.03 (Table 1).

**Table 1:** Overview of estimated pooled effect sizes of growth factor moderators using a three level meta-analysis random effects model.

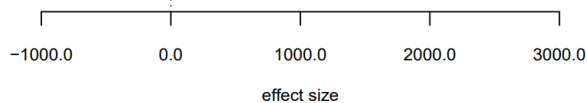
<b>Moderator</b>	<b>Estimate</b>	<b>Standard error</b>	<b>T value</b>	<b>Degrees of freedom</b>	<b>P value</b>	<b>Lower confidence interval</b>	<b>Higher confidence interval</b>
ECM	17.1264	166.6966	0.1027	180	0.9183	-311.8045	346.0573
Soil type	74.3559	34.3555	2.1643	199	0.0316	6.6084	140
Growth conditions	44.2207	43.3697	1.0196	202	0.309	-41.2948	129.73
Tree age	31.227	32.1408	0.9716	202	0.3324	-32.1475	94.6015
Tree genus	71.552	55.2014	1.2962	202	0.1964	-32.2927	180.3971

Study	Estimate [95% CI]
Svenson, 1991	-10.0   -14.0, -6.0
Svenson, 1991	0.2   -3.9, 4.2
Shaw, 1995	-1.5   -4.4, 1.4
Shaw, 1995	0.1   -2.8, 3.0
Scagel, 1998	-0.2   -1.2, 0.9
Scagel, 1998	0.0   -1.0, 1.0
Scagel, 1998	-0.4   -1.6, 0.8
Scagel, 1998	0.0   -1.0, 1.1
Scagel, 1998	0.1   -0.9, 1.1
Scagel, 1998	1.1   -0.4, 2.7
Scagel, 1998	-0.1   -1.9, 1.7
Scagel, 1998	0.1   -1.6, 1.9
Scagel, 1998	-0.8   -2.8, 1.3
Scagel, 1998	-0.1   -2.0, 1.7
Scagel, 1998	-0.5   -2.4, 1.5
Scagel, 1998	-0.0   -1.2, 1.1
Scagel, 1998	-0.4   -1.7, 0.9
Scagel, 1998	0.1   -1.1, 1.2
Scagel, 1998	-0.1   -1.3, 1.1
Scagel, 1998	0.0   -1.1, 1.1
Scagel, 1998	0.1   -1.0, 1.2
Scagel, 1998	-1.8   -3.6, 0.1
Scagel, 1998	-1.4   -3.1, 0.3
Scagel, 1998	0.3   -0.7, 1.2
Scagel, 1998	0.0   -1.0, 1.0
Scagel, 1998	-1.2   -2.8, 0.4
Scagel, 1998	0.0   -1.8, 1.8
Scagel, 1998	0.0   -1.1, 1.1
Scagel, 1998	0.4   -0.7, 1.5
Scagel, 1998	0.3   -0.8, 1.4
Scagel, 1998	-0.2   -1.4, 1.1
Scagel, 1998	0.2   -1.0, 1.3
Scagel, 1998	-0.1   -1.3, 1.1
Scagel, 1998	0.3   -1.0, 1.5
Scagel, 1998	0.3   -0.9, 1.5
Scagel, 1998	0.2   -1.0, 1.4
Scagel, 1998	0.7   -0.5, 1.8
Scagel, 1998	0.6   -0.6, 1.7
Scagel, 1998	0.2   -1.0, 1.5
Scagel, 1998	-0.2   -1.6, 1.3
Scagel, 1998	-2.4   -6.0, 1.2
Probanza, 2001	33.2   29.6, 36.9
Probanza, 2001	12.2   8.6, 15.8
Niemi, 2002	-0.9   -2.1, 0.3
Niemi, 2002	-0.9   -2.2, 0.3
Niemi, 2002	-1.7   -3.0, -0.4
Niemi, 2002	0.4   -0.8, 1.6
Niemi, 2002	-0.6   -4.9, 3.8
Boukcim, 2003	21.5   17.1, 25.8
Boukcim, 2003	-12.0   -16.4, -7.7
Guerin-Laguet, 2003	14.8   12.3, 17.2
Guerin-Laguet, 2003	13.3   10.9, 15.8
Guerin-Laguet, 2003	5.0   2.7, 7.4
Guerin-Laguet, 2003	11.5   9.0, 13.9
Guerin-Laguet, 2003	0.0   -2.2, 2.2
Mari, 2003	-27.6   -31.4, -23.9
Hilszczanska, 2004	-3.6   -7.4, 0.1
Duñabeitia, 2004	17.7   12.6, 22.8
Duñabeitia, 2004	61.6   56.5, 66.7
Duñabeitia, 2004	16.0   10.9, 21.1
Duñabeitia, 2004	0.0   -5.1, 5.1
Duñabeitia, 2004	45.6   40.5, 50.7
Duñabeitia, 2004	53.7   48.6, 58.8
Duñabeitia, 2004	2.3   -2.8, 7.4
Duñabeitia, 2004	12.8   7.7, 17.9
Duñabeitia, 2004	15.9   10.8, 21.0
Duñabeitia, 2004	11.8   6.7, 16.9
Duñabeitia, 2004	5.2   0.1, 10.3
Duñabeitia, 2004	17.1   12.0, 22.2
Choi, 2005	13.1   8.6, 17.5
Rincón, 2005	-72.0   -77.1, -66.8
Rincón, 2005	12.0   6.9, 17.1
Bakker, 2006	2.3   0.1, 4.5
van Hees, 2006	-0.1   -1.5, 1.4
van Hees, 2006	0.9   -0.4, 2.2
Rincón, 2007	-4.4   -7.1, -1.8
Rincón, 2007	6.3   3.6, 8.9
Rincón, 2007	5.8   3.1, 8.4
Rincón, 2007	9.6   6.9, 12.2
Rincón, 2007	8.1   5.5, 10.8
Aucina, 2007	28.2   23.1, 33.3
Kozdrój, 2007	2.3   0.8, 3.9
Kozdrój, 2007	2.3   0.7, 3.8
Kozdrój, 2007	11.0   8.8, 13.1
Kozdrój, 2007	10.7   8.5, 12.8
Niemi, 2007	186.3   181.8, 190.7
Niemi, 2007	276.4   271.8, 281.0
Niemi, 2007	1.0   -0.3, 2.3
Niemi, 2007	1.0   -0.3, 2.3
Zhu, 2008	14.4   11.9, 16.8
zhu, 2008	0.7   -1.5, 2.9
Zhu, 2008	12.1   9.8, 14.5
Zhu, 2008	11.4   9.1, 13.8
Zhu, 2008	13.7   11.3, 16.1
Zhu, 2008	4.2   2.0, 6.4



Heterogeneity:  $I^2_{level3} = 23.27\%$ ,  
 $I^2_{level2} = 76.73\%$ ,  $P < 0.0001$

Estimate: 51, 95%CI: 1.73- 100,  $p = 0.04$



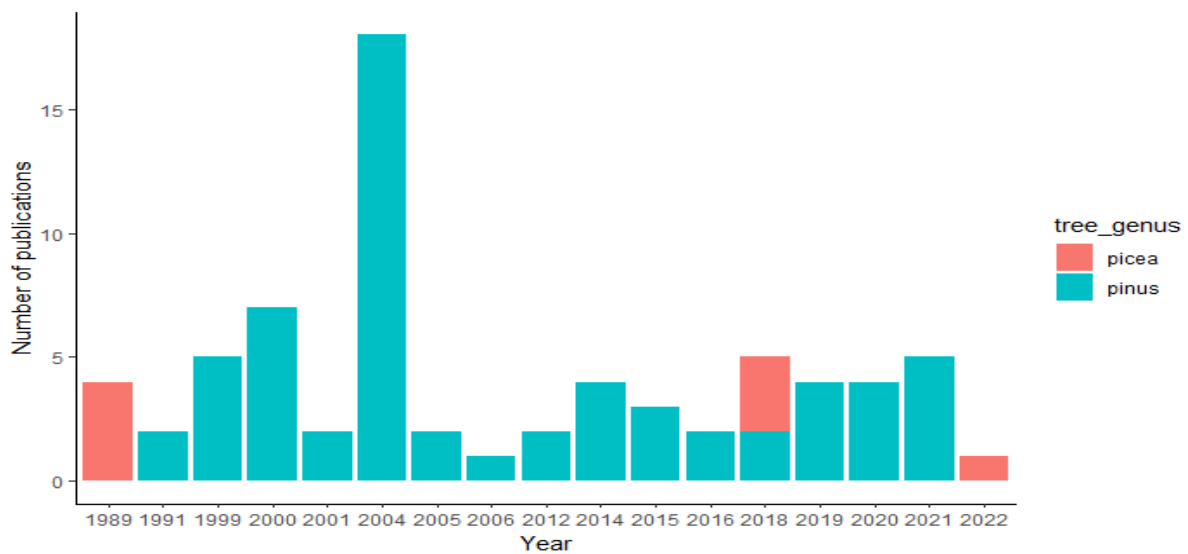


**Figure 9:** Forest plot of the overall effects of ECM on growth

Forest plot of the overall effects of Ectomycorrhizal fungi on *Pinus* and *Picea* growth. Error bars represents 95% confidence intervals (CI). The black diamond on the scale is representing the overall effect of the study.

### 3.2 Effect of ECM on conifer resistance to abiotic stress

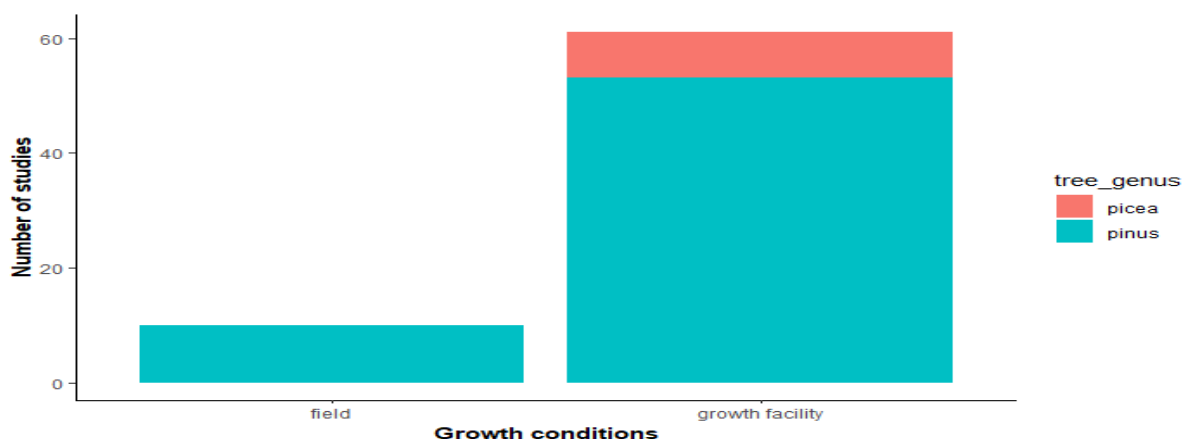
In contrary to *Pinus* species, from the year 1991 to 2022, there are scanty recorded studies of *Picea* species (only recorded three times). The total number of articles was high in 2004, 2007 and 2012, followed by a substantial decrease in 2005 and later.



**Figure 9:** Number of publications on conifer resistance to abiotic stress

Publication year of articles included in the meta-analysis of ECM effect on *Pinus* and *Picea* on abiotic stress.

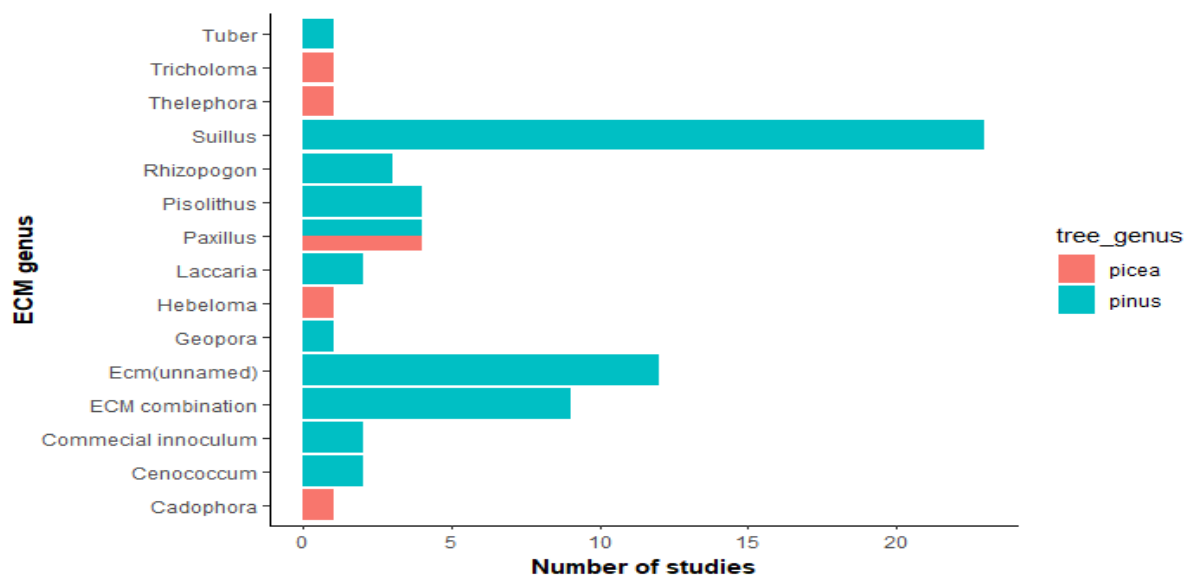
Growth studies were mostly conducted in a growth facility, and much of the investigation was conducted on *Pinus*. No field studies were conducted on *Picea* (Figure 9).



**Figure 10:** Growth conditions under which studies on abiotic stress in the meta-analysis were conducted.

Growth condition under which studies included in meta-analysis of ECM effect on *Pinus* and *Picea* on abiotic stress were conducted. Studies conducted under controlled environments, greenhouse, glasshouse, lab, or nursery, were allocated to “growth facility” and those which were carried out in the field were allocated to “field”.

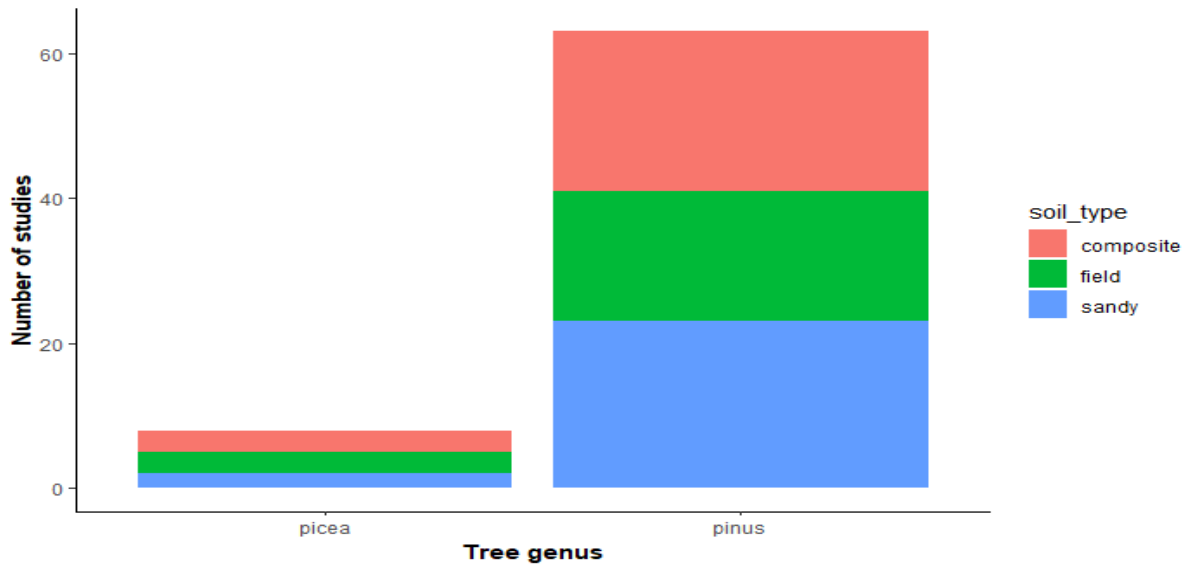
The most common ECM genera used were members of the genus *Suillus* or a combination of different ectomycorrhizal fungi. The most tested tree species were in the genus *Pinus*. (Figure 10).



**Figure 11:** ECM genera which were used for growth studies in the meta-analysis.

ECM genera used in studies included in meta-analysis of ECM effect on *Pinus* and *Picea* on abiotic stress were conducted. ECM denotes ectomycorrhizal fungi where more than one ECM genus was used.

Soil types: composite, field and sandy were used on both *Pinus* and *Picea* and media soil type was not used on both tree species. The number of studies performed using these three soil types were almost equal on *Pinus* species and on *Picea* species as well (Figure 12).

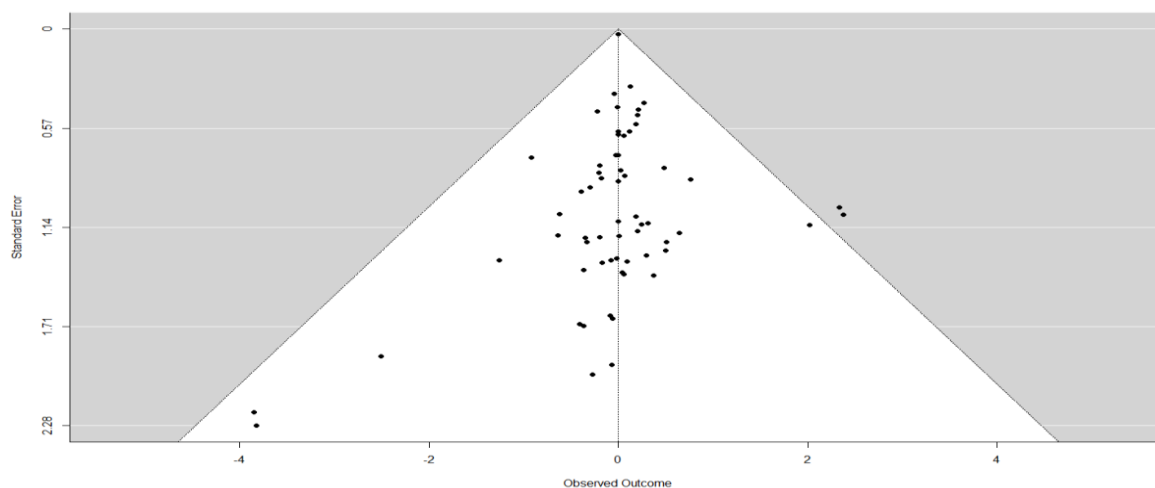


**Figure 12:** Soil type used on conifer resistance to abiotic stress studies.

Soil from the forest or field was categorised as field. Nursery soils, i.e., various mixtures of soil substrates such as vermiculite and peat, were categorised as composite soil. Studies using sand as the main substrate were classified as sandy soil. Liquid substrates and agars were classified as media.

The overall pooled effect size based on the three-level meta-analysis model was 0.0014 (95% CI: -0.02- 0.02;  $p=0.9$ ), indicating a very small effect of ectomycorrhizal fungi on abiotic stress. There is low level of heterogeneity in the abiotic factor.  $I^2_{\text{level } 3}$  (overall effect size) was 0% and  $I^2_{\text{level } 1}$  (individual effects) was 100%, meaning variability in effect size estimates is due to sampling error within studies. (Figure 12).

There is an indication of publication bias because small studies that favours ectomycorrhizal fungi are missing at the lower right hand-side of the plot vice versa (the plot is not symmetrical) or small negative studies at the bottom left do not balance small positive studies at the bottom right. However almost all studies are not significant as only 2 studies were significant.



**Figure 13:** Funnel plot showing publication bias on abiotic stress studies.

Funnel plot showing publication bias of abiotic stress studies. Each dot represents a study (measuring effect of ectomycorrhizal fungi). The y-axis represents the study precision (standard error) and the x-axis shows the study outcome. The outer dashed lines show the 95% Confidence Interval limit. The average effect size is shown by the dashed line in the middle. Larger and most powerful studies are placed towards the top. In the absence of a bias the scatter will resemble a symmetrical inverted funnel.

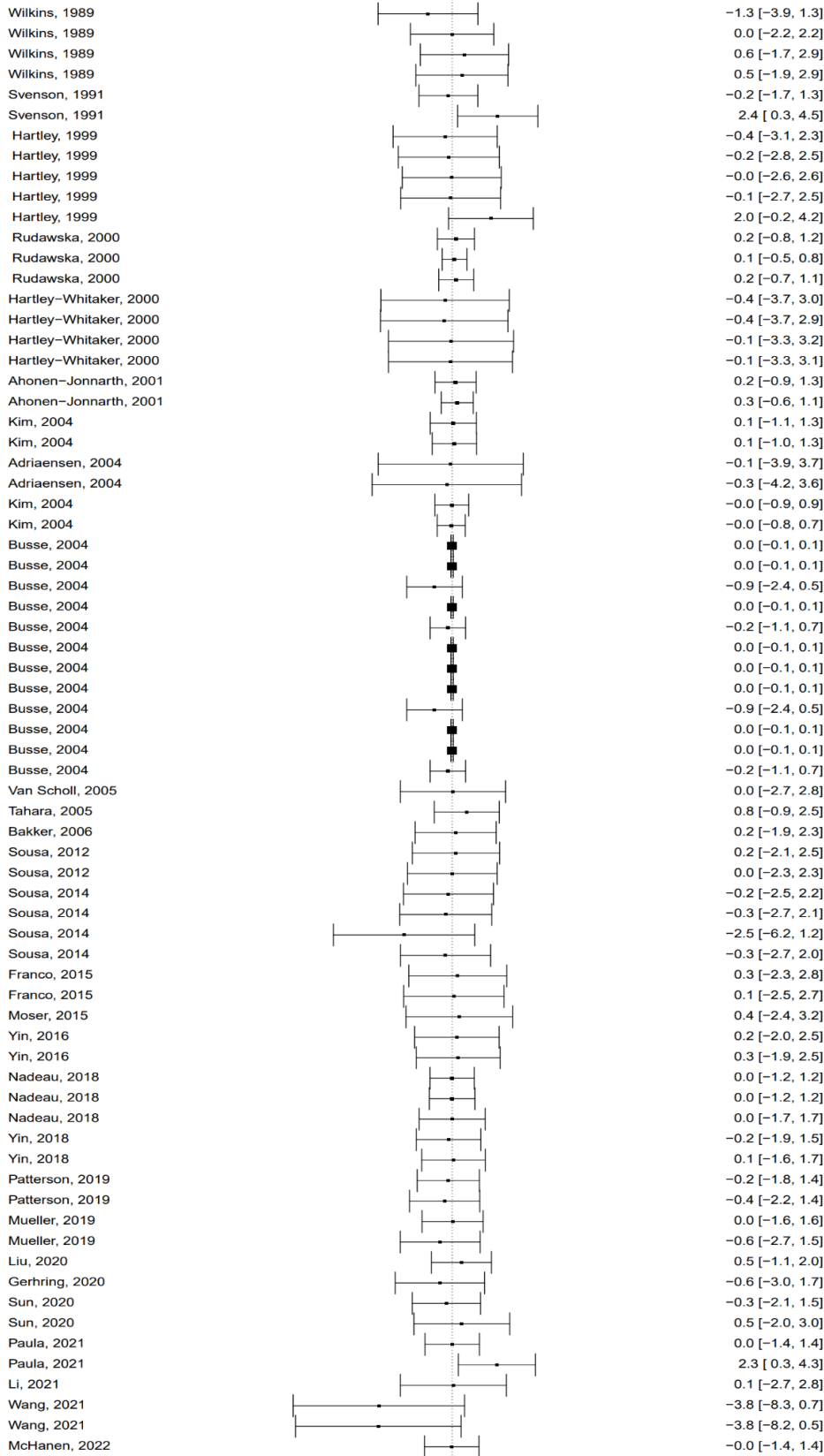
The pooled estimated effects sizes of the moderators were dispersed around zero meaning ectomycorrhizal fungi did not enhance conifer resistance to abiotic stress (Table 2). They had no significant moderating effect ( $P > 0.05$ ) on the relationship between ectomycorrhizal fungi and conifer resistance to abiotic stress.

**Table 2:** Overview of estimated pooled effect sizes of abiotic factor moderators using a three level meta-analysis random effects model.

Moderator	Estimate	Standard error	T value	Degrees of freedom	P value	Lower confidence interval	Higher confidence interval
ECM	0.001	0.5904	0.0017	56	0.9987	-1.1818	1.1838
Soil type	0.079	0.1356	0.5829	68	0.5619	-0.1915	0.3496
Growth conditions	0.136	0.3183	0.4272	69	0.6705	-0.499	0.7709
Tree age	0.0057	0.0375	0.152	69	0.8796	-0.0691	0.0805
Tree genus	0.0047	0.2939	-0.0159	69	0.9874	-0.5816	0.5909
Stress type	-0.527	0.2597	-0.2029	68	0.8398	-0.5709	0.4655

Study

Estimate [95% CI]



Heterogeneity:  $I^2_{level 3} = 0\%$ ,  $I^2_{level 1} = 100\%$ ,  $p = 1$

Estimate: 0.0014 (95%CI: -0.02- 0.02;  $p=0.9$ )

-10.0 -5.0 0.0 5.0  
effect size

**Figure 14:** Forest plot of the overall effects of ECM on conifer resistance to abiotic stress

Forest plot of the overall effects of ectomycorrhizal fungi on *Pinus* or *Picea* to abiotic stress. Error bars represents 95% confidence intervals (CI). The black diamond on the scale is representing the overall effect of the study.

### 3.3 Effects of ECM on conifer tree Survival

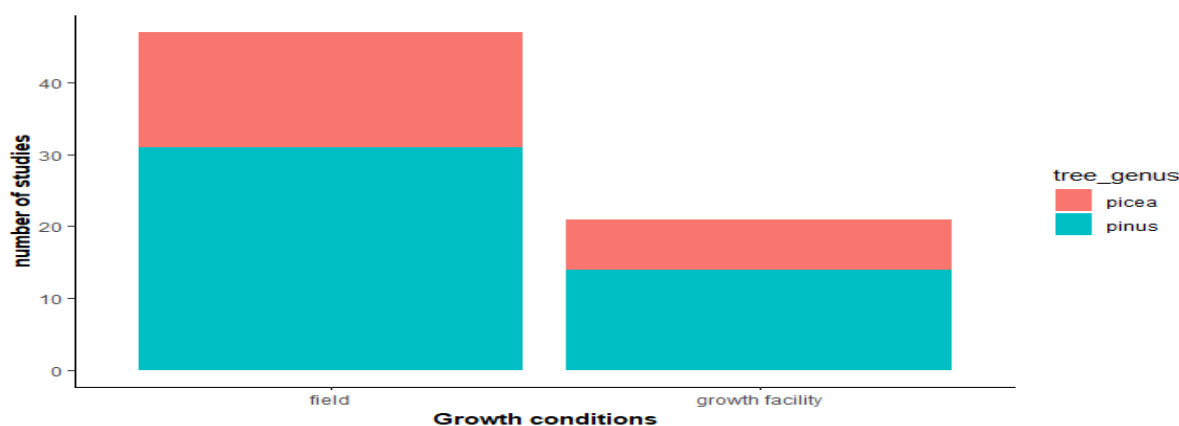
There are more published articles on *Pinus* compared to *Picea*. Publications for *Picea* were high in 2005 and 2011 followed by a sharp decrease in 2014. There were no *Picea* publications between 2014 to 2020 (Figure 13).



**Figure 15:** Number of publications on conifer survival.

Publication year of articles included in the meta-analysis of ECM effect on *Pinus* and *Picea* survival.

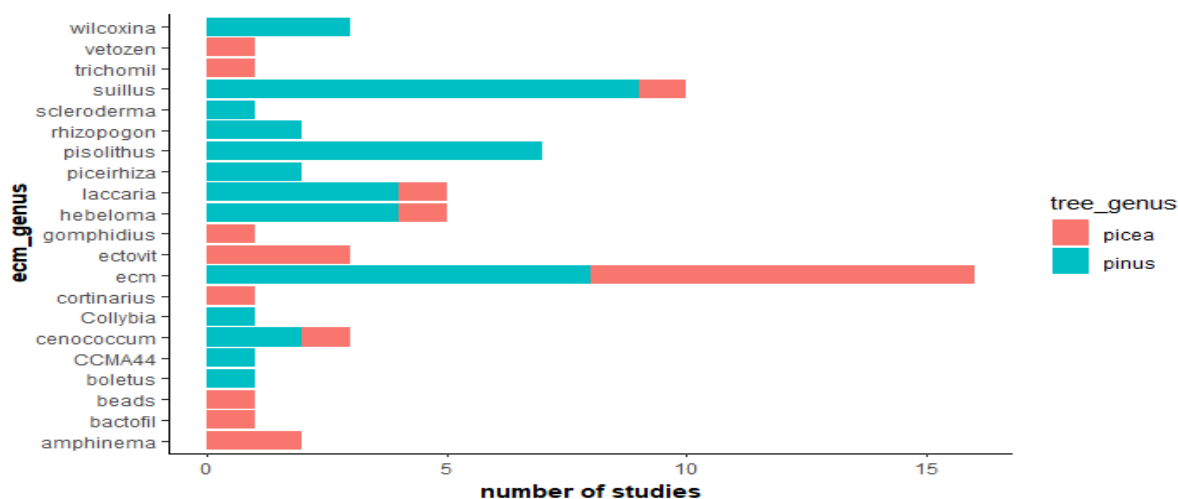
Most studies were conducted in the field and a significant number of *Picea* species were tested in the field (Figure 14).



**Figure 16:** Growth conditions under which studies on conifer survival in the meta-analysis were conducted.

Growth condition under which studies included in meta-analysis of ECM effect on *Pinus* and *Picea* survival were conducted. Studies conducted under controlled environments, greenhouse, glasshouse, lab, or nursery, were allocated to “growth facility” and those which were carried out in the field were allocated to “field”.

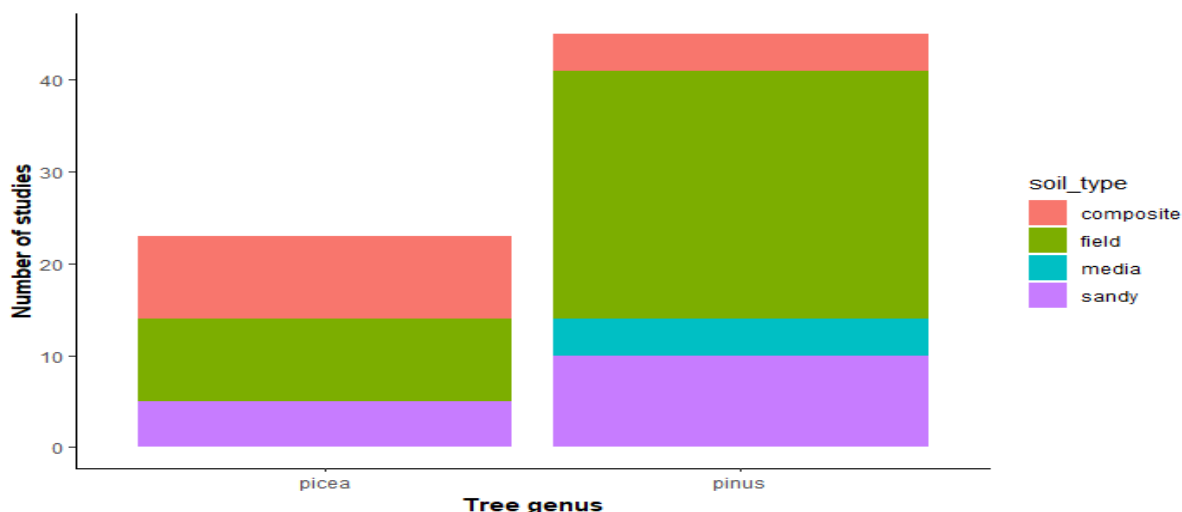
The most common ECM genera used were *Suillus*, *ECM combination* and *Pisolithus*. ECM was mostly tested on *Pinus* species (Figure 15).



**Figure 17:** ECM genera which were used for growth studies in the meta-analysis.

ECM genera used in studies included in meta-analysis of ECM effect on *Pinus* and *Picea* on survival were conducted. ECM denotes ectomycorrhizal fungi where more than one ECM genus was used.

All the four soil types were used on *Pinus* species and three soil types excluding media were used on *Picea* species. The most used soil type on *Pinus* species was the field soil. Both field and composite soil types were mostly used on *Picea* species.

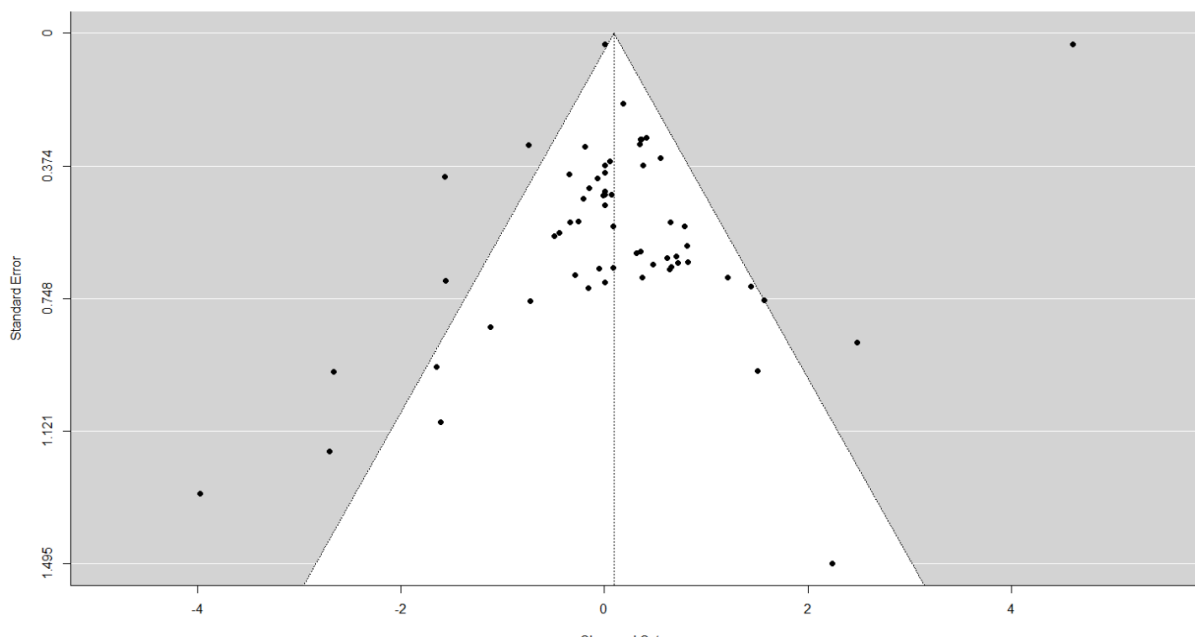


**Figure 18:** Soil type used on conifer tree survival studies.

Soil from the forest or field was categorised as field. Nursery soils, i.e., various mixtures of soil substrates such as vermiculite and peat, were categorised as composite soil. Studies using sand as the main substrate were classified as sandy soil. Liquid substrates and agars were classified as media.

The overall pooled estimated effect size based on the three-level meta-analysis model was 0.09 (95% CI: 0.25- 0.44;  $p = 1$ ) meaning that ectomycorrhizal fungi had a negative effect on conifer survival. There is high heterogeneity in the study,  $I^2_{\text{level 3}}$  (overall effect size) was 34.27% and  $I^2_{\text{level 2}}$  (nested effect sizes from individual studies) was 64.61% (Figure 17).

There is an indication of publication bias because the graph is asymmetrical. Small studies that do not favour and that favours ectomycorrhizal fungi are missing at the lower right hand and left hand of the plot. Most studies are not significant, and a few significant studies are outside the triangle.



**Figure 19:** Funnel plot showing publication bias of survival studies.

Funnel plot showing publication bias of studies on survival. Each dot represents a study (measuring effect of ectomycorrhizal fungi). The y-axis represents the study precision (standard error) and the x-axis shows the study outcome (effect size). The outer dashed lines show the 95% confidence interval. The average effect size is shown by the dashed line in the middle. Larger and most powerful studies are placed towards the top. In the absence of a bias the scatter will resemble a symmetrical inverted funnel.



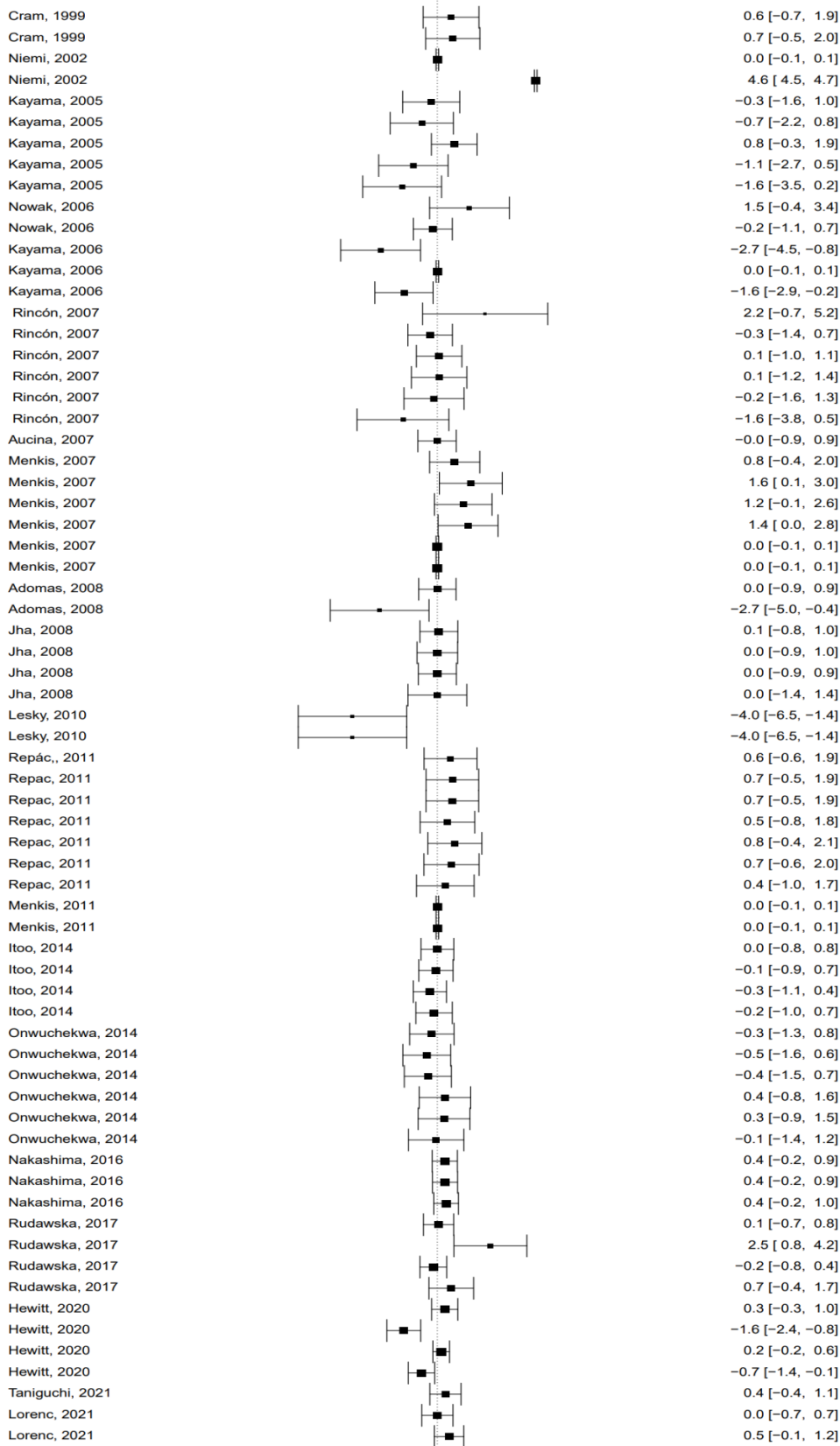
The pooled estimated effects sizes of the moderators were dispersed around zero meaning ectomycorrhizal fungi did not enhance and conifer tree survival (Table 3). They had no significant moderating effect ( $P>0.05$ ) on the relationship between ectomycorrhizal fungi and conifer survival.

**Table 3:** Overview of estimated pooled effect sizes of survival factor moderators using a three level meta-analysis random effects model.

<b>Moderator</b>	<b>Estimate</b>	<b>Standard error</b>	<b>T value</b>	<b>Degrees of freedom</b>	<b>P value</b>	<b>Lower confidence interval</b>	<b>Higher confidence interval</b>
ECM	0.001	0.6831	0.0015	47	0.9988	-1.3732	1.3752
Soil type	-0.0876	0.4044	-0.2165	63	0.8293	-0.8957	0.7206
Growth conditions	0.0654	0.219	-0.2986	66	0.7662	-0.3719	0.5027
Tree age	0.2698	0.3117	0.8657	66	0.3898	-0.3525	0.8921
Tree genus	0.1403	0.3002	0.4672	66	0.6419	-0.4592	0.7397

Study

Estimate [95% CI]



Heterogeneity:  $I^2_{level3} = 34.27\%$ ,  
 $I^2_{level2} = 64.61\%$ ,  $P < 0.0001$

Estimated effect = 0.09, 95% CI: -0.25-  
 0.44;  $p = 1$

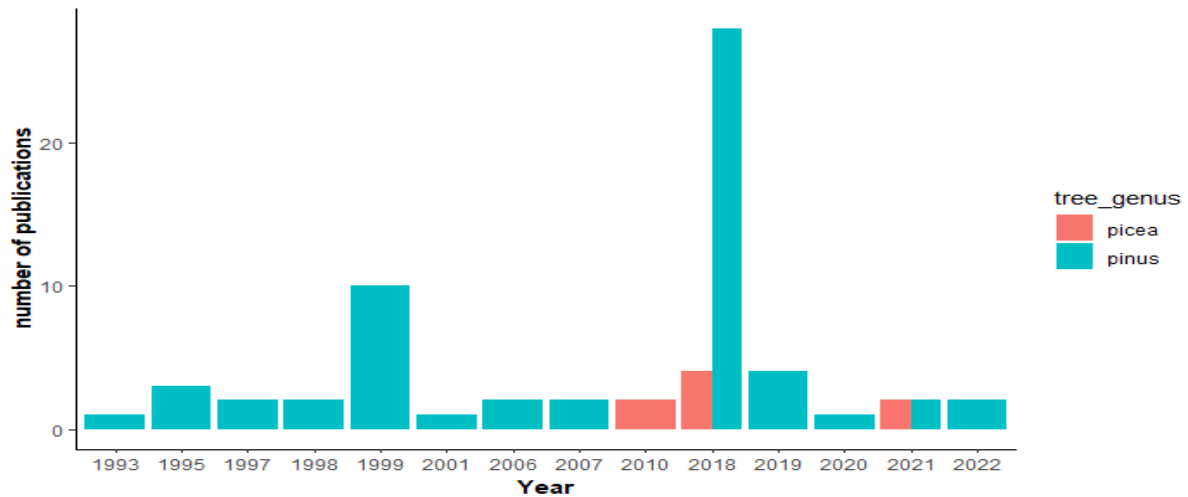
-10.0      -5.0      0.0      5.0      10.0  
 effect size

**Figure 20:** Forest plot of the overall effects of ECM on conifer survival

Forest plot of the overall effects of ectomycorrhizal fungi on *Pinus* or *Picea* survival. Error bars represents 95% confidence intervals (CI). The black diamond on the scale is representing the overall effect of the study.

### 3.4 Effects of ECM on conifer resistance to biotic stress

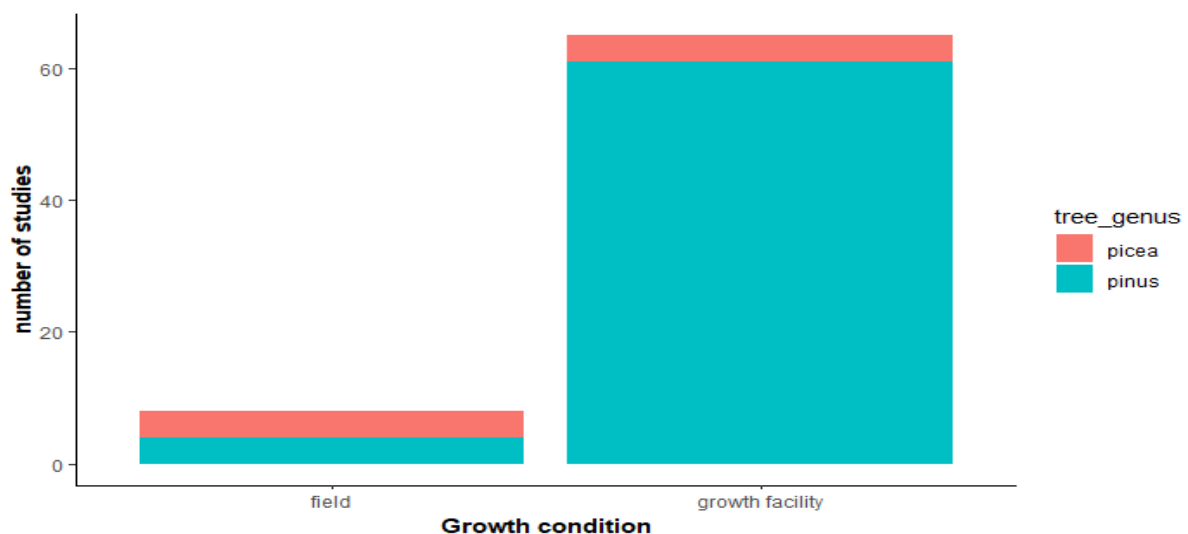
There were very few publications on *Picea* species between 1993 and 2022. Only three articles were published (2010, 2018 and 2021), (Figure 20).



**Figure 21:** Number of publications on conifer resistance to biotic stress

Publication year of articles included in the meta-analysis of ECM effect on *Pinus* and *Picea* on biotic stress.

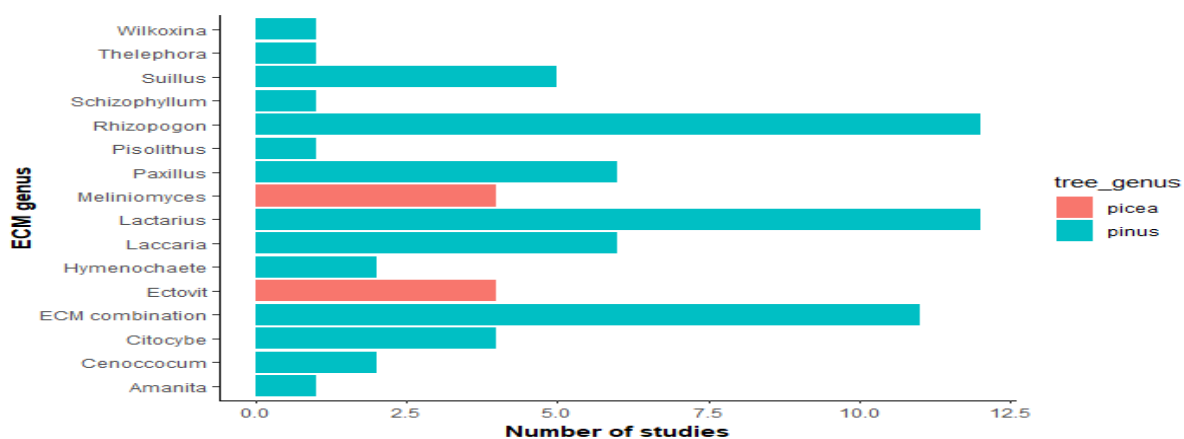
Most studies were conducted in a growth facility with *Pinus* accounting to more than half of the studies. Minute studies were conducted in the field on both tree species.



**Figure 22:** Growth conditions under which studies on conifer biotic stress studies in the meta-analysis were conducted.

Growth condition under which studies included in meta-analysis of ECM effect on *Pinus* and *Picea* on biotic stress were conducted. Studies conducted under controlled environments, greenhouse, glasshouse, lab, or nursery, were allocated to “growth facility” and those which were carried out in the field were allocated to “field”. (*Pinus* is represented by blue and *Picea* by pink).

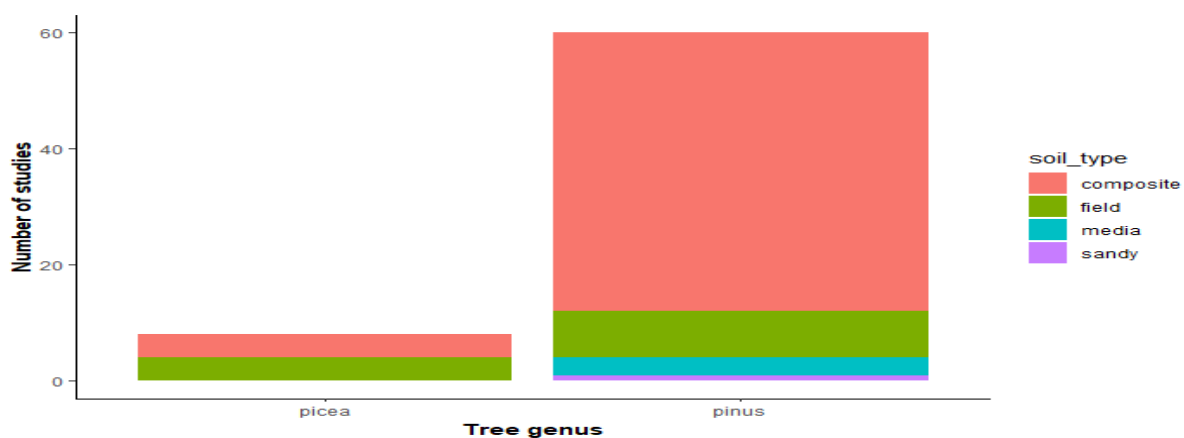
The most used ECM were the *Rhizopogon*, *Lactarius*, *Suillus*, *Paxillus* and ECM combination, but they were only experimented *Pinus*. There were very few studies on *Picea* species (Figure 20).



**Figure 23:** ECM genera which were used for biotic studies in the meta-analysis.

ECM genera used in studies included in meta-analysis of ECM effect on *Pinus* and *Picea* on biotic stress were conducted. ECM denotes ectomycorrhizal fungi where more than one ECM genus was used.

The most common used soil type on *Pinus* species was the composite followed by the field soil type. Both composite and field soil types were equally used on *Picea* species.



**Figure 24:** Soil type used on conifer resistance to biotic stress studies

Soil from the forest or field was categorised as field. Nursery soils, i.e., various mixtures of soil substrates such as vermiculite and peat, were categorised as composite soil. Studies using sand as the main substrate were classified as sandy soil. Liquid substrates and agars were classified as media.

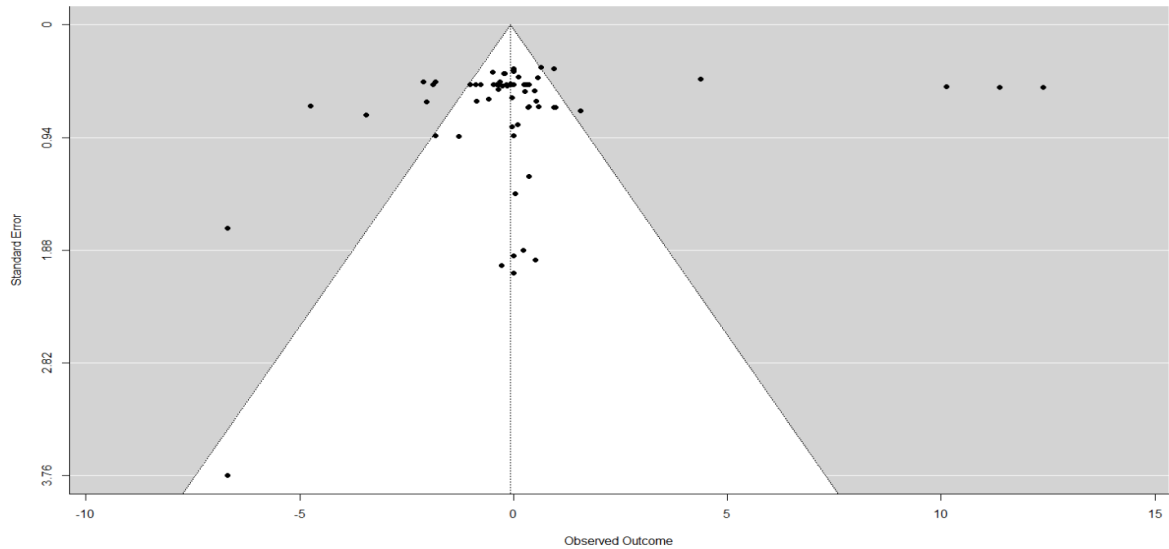
The overall pooled estimated effect size based on the level three meta-analysis model was (-0.1), (95% CI: -0.8- 0.69;  $p = 1$ ) meaning ectomycorrhizal fungi did not improve tree resistance to biotic stress. There is high heterogeneity in the biotic factor study.  $I^2_{\text{level 3}}$  (overall effect size) was 5.5% and  $I^2_{\text{level 2}}$  (nested effect sizes from individual studies) was 90.34% (Figure 22).

The pooled estimated effects sizes of the moderators were below zero indicating a very weak relationship on biotic stress (Table 4). They had no significant moderating effect ( $P > 0.05$ ) on the relationship between ectomycorrhizal fungi and conifer resistance to biotic stress, though growth conditions, tree age and soil type had positive effects.

**Table 4:** Overview of estimated pooled effect sizes of biotic factor moderators using a three level meta-analysis random effects model.

Moderator	Estimate	Standard error	T value	Degrees of freedom	P value	Lower confidence interval	Higher confidence interval
ECM	-2.078	0.6831	0.0015	56	0.4721	-7.6393	3.6873
Soil type	-0.1581	0.4044	-0.2165	63	0.8697	-2.0762	1.7599
Growth conditions	0.8807	0.219	-0.2986	71	0.3722	-2.8363	1.0749
Tree age	0.9474	0.3117	0.8657	57	0.7386	-4.7106	6.6054
Stress type	7.7104	5.4015	1.4275	37	0.1618	-3.2341	18.6549

There is an indication of publication bias because the graph looks asymmetrical. There is only one small study that does not favour ectomycorrhizal fungi at the lower bottom left of the funnel (missing small studies). Most studies are however not significant.

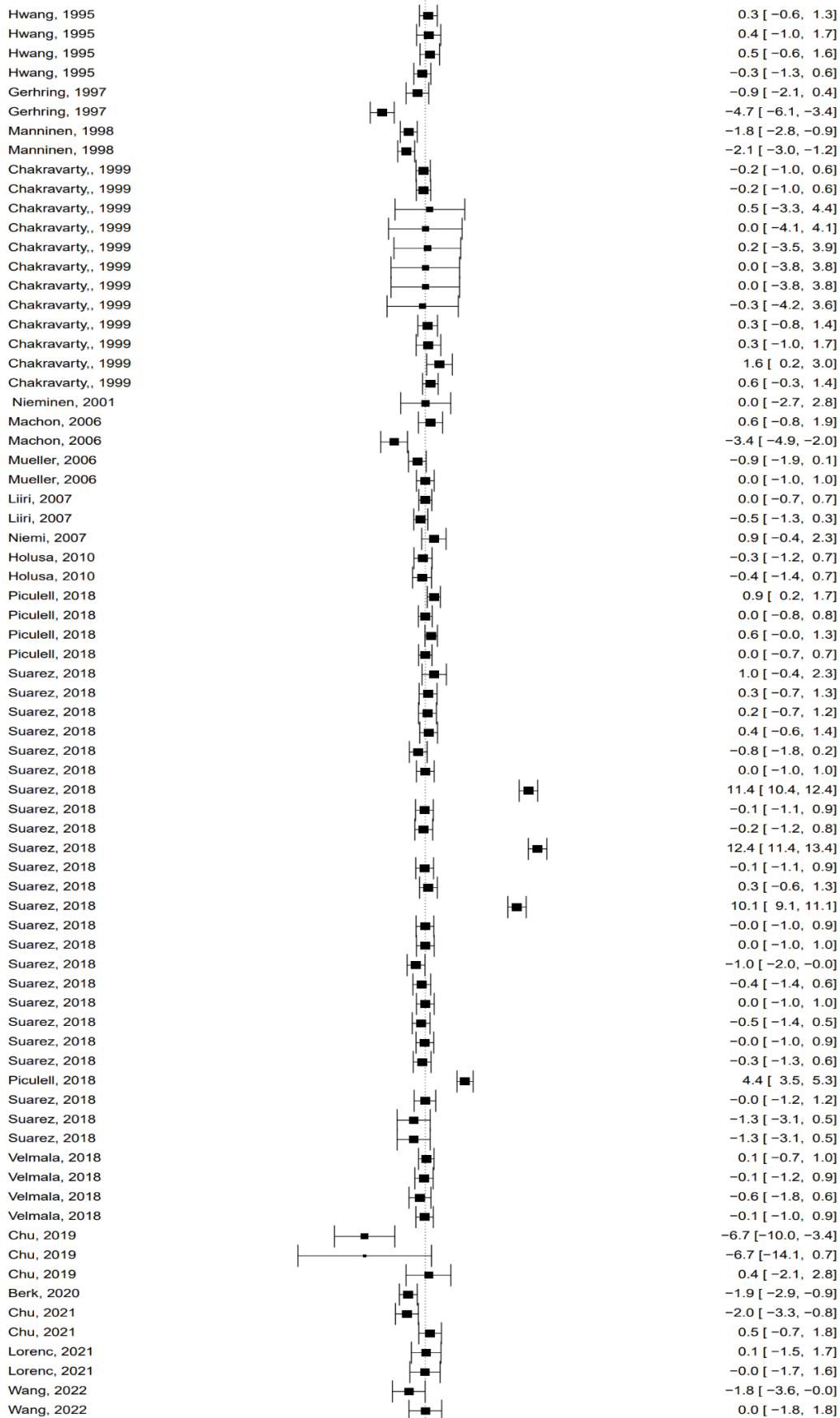


**Figure 25:** Funnel plot showing publication bias of biotic studies.

Funnel plot showing publication bias of biotic studies. Each dot represents a study (measuring effect of ectomycorrhizal fungi). The y-axis represents the study precision (standard error) and the x-axis shows the study outcome. The outer dashed lines shows the 95% confidence interval limit. The average effect size is shown by the dashed line in the middle. Larger and most powerful studies are placed towards the top. In the absence of a bias the scatter will resemble a symmetrical inverted funnel.

Study

Estimate [95% CI]



Heterogeneity:  $I^2_{level3} = 5.5\%$ ,  $I^2_{level2} = 90.34\%$ ,  
 $I^2_{level2} = 4.97\%$ ,  $P < 0.0001$

Estimate: -0.1, 95% CI: -0.8-0.69,  
 $p = 1$

-15.0 -5.0 0.0 5.0 10.0 15.0  
 effect size

### Figure 17:

Forest plot of the overall effects of ectomycorrhizal fungi on *Pinus* or *Picea* to biotic stress. Error bars represents 95% confidence intervals (CI). The black diamond on the scale is representing the overall pooled effect size of the studies.

## 4.0 DISCUSSION

In this study a meta- analysis of the effects of ectomycorrhizal fungi in enhancing conifer (*Pinus* and *Picea*) growth, survival and resistance to biotic and abiotic stress was conducted. I found that there was a large body of literature on this topic (1806 publications). Statistical data was recorded for articles which measured morphological characteristics such as seedling height, root length and stem diameter. This is because plant height is closely related to the life span, seed mass and time to maturity(Moles et al., 2009), and linked to the yield potential(Tilly et al., 2014). Stem diameter has been considered to be the best predictor of field survival and growth (Haase, 2008) and root length very important because it controls water and nutrient uptake. Other parameters recorded were growth rate, survival, mortality and disease incidence. Several articles which were published in the 1980s and 1990s were in accessible, thus were excluded for the meta-analysis. The reason may be because they had not been digitalised during that time.

A meta-analysis outcome can be affected by publication and other forms of selection biases, and funnel plots are usually used to quantify and detect such biases (Tang & Liu, 2000). A symmetrical funnel indicates absence of bias, and an asymmetrical funnel indicates bias(Sterne & Harbord, 2004), (Figure 5). There were huge volume of studies on growth which may have caused the width of the confidence intervals to decrease(Higgins, 2011). There is an indication of publication bias in this study which is indicated by the asymmetry of funnel plots(Sterne & Harbord, 2004). However asymmetrical shape of the funnel plots may also be as a result of other biases such as non- inclusion of articles that were not accessible but relevant, language (only studies written in English was included), method of selection or small study effects. Additional, negative studies have a small chance on publication in English language journals, although the regularity has not always been observed(Thornton & Lee, 2000). The bias can also be as a result of citation as studies with positive results are cited more. As a result, they are easily identified and incorporated in databases (Eyding et al., 2010). Karst et al. (2023) also gave evidence of citation bias on mycorrhizal networks in forests and this has affected a clear understanding of mycorrhizal structure and function.



This analysis is also characterised by high heterogeneity which made it difficult to interpret the results. Higgins and Thompson (2002) argue that heterogeneity is inevitable and any level of heterogeneity is acceptable provided that the studies included were appropriate and the data was handled properly. In this analysis, I used the widely accepted heterogeneity statistic  $I^2$ . However it should be noted that this statistic may overestimate the heterogeneity when the sample size of the study is small. (von Hippel, 2015). Heterogeneity between studies may also produce asymmetrical funnel plots. The plot assumes that the spread of effect sizes is as a result of heterogeneity, but this does not necessarily indicate that the effects are insignificant. Additionally, when dealing with larger studies more investment is needed and there is a chance of the methodology being more rigorous. This can also contribute to asymmetry in the funnel plots (Page et al., 2020). Thus, all conclusions made in this thesis should be considered with these biases in mind.

Lack of correct identification of mycorrhiza may also contribute to inaccurate results in that species may have been incorrectly designated in databases. This is supported by Fruleux et al. (2022) who argues that there may be putative diagnosis errors such as reported ectomycorrhizal fungi which will be otherwise Arbuscular mycorrhizal fungi. Improving sequencing technologies that produce more longer sequence reads and more accurate base calling may help to identify mycorrhiza and resolve these inaccuracies.

In this study, most of the experiments were conducted in growth facilities except for survival studies where field experiments dominated. This limited transfer of methods to the field was highlighted as critical regardless of publication bias by Khokon and Meier (2023). The criticality comes in the sense that the field encompasses the widest ecosystem where mycorrhizal traits could be studied without moderating environmental conditions. Lack of consistency in the results of this study was also as a result of lack of more published articles on the relationship of ECM with *Picea* or *Pinus*. The whole study was also dominated by the use of *Suillus* species as the ectomycorrhizal inoculant. A total of 8.2% of ectomycorrhizal fungi papers in the past 40 years have concerned *Suillus* which is known to exhibit a high degree of specificity to conifers (Dahlberg & Finlay, 1999). Interestingly Zhang et al. (2022) study on host shift speciation of *Suillus* and the Pinaceae did not identify any cospeciation patterns between *Suillus* and the sub genera Pinacea and their history together is seen to be discordant.

The initial research question of this meta-analysis was to know if there is evidence of ectomycorrhizal fungi in enhancing conifer growth and survival and if it improves their resistance to biotic and abiotic stress. Ectomycorrhizal fungi were however found to enhance growth but no significant effect was found on enhancing conifer survival and resistance to biotic and abiotic stress. The evidence of ectomycorrhizal fungi in enhancing growth is supported in a meta-analysis by Alberton et al. (2014) where ECM was found to increase growth of *Pinus species*. However, data from only 6 studies were collected for this meta-analysis. A meta-analysis by Gan et al. (2021) provided some quantitative evidence of the rhizosphere in influencing below ground carbon and nutrient cycling in forest ecosystems which in turn improves growth.

In this study, ectomycorrhizal fungi were however found to have no relationship on tree resistance to abiotic stress. A review by Lehto and Zwiazek (2011) suggested that mycorrhizal structure may impede water movement to the plant due to fine root architecture or the hydrophobicity of the cell wall. This may heavily affect the plant's water absorption efficiency and can get worse during drought stress. Defrenne et al. (2019) research revealed a distinct root structure from seedlings planted in a nursery compared to seedlings regenerated in the field. This architectural difference was thought to lack of ectomycorrhizal partners, which may in turn affect a plant's response to abiotic stress, survival and growth.

Karst et al. (2008) used a meta-analysis to quantitatively evaluate the role of biotic and abiotic factors on host growth and responses to ectomycorrhizal associations on *Pinus* and *Picea*. Overall, in their analysis the host biomass increased in response to ectomycorrhizal inoculation, but the results were distorted by publication bias and methodological issues thus, distorted the spectrum on which they evaluated the host responses to ectomycorrhizal inoculation. This also supports this meta-analysis.

In this study ectomycorrhizal fungi had no effect on survival. This is revealed in Quoreshi et al. (2008), where conifer survival rate of the inoculated seedlings was not significant from the control whose seedlings were naturally colonised by the resident fungi. Nevertheless, ectomycorrhizal fungi helped the conifers to alleviate biotic stress against pathogens and insects. A meta-analysis by Holden and Treseder (2013), showed an increase of fungal abundance following an insect infestation and pathogen induced mortality in a boreal forest.

Howbeit, fungal abundance does not imply the effectiveness of ectomycorrhizal fungi to the host plant (Wagg et al., 2011).

In this study moderators such as soil type, growth condition and tree age were tested to determine their influence on the relationship between conifer growth, survival and resistance to biotic and abiotic stress and ectomycorrhizal fungi. All the moderators They had no significant moderating effect on the relationship between ectomycorrhizal fungi and conifer tree resistance to biotic stress, survival and growth, except for soil type as whole. Therefore, the study cannot determine which soil type contributed the most because they did not have a significant effect when analysed individually but had a significant effect when analysed as a whole.

## **5.0 CONCLUSION**

The interaction between ectomycorrhizal fungi and their conifer host is very complex. Thus it is difficult to capture all the factors that influence the relationship within a study. In this study, publication bias and study heterogeneity made it difficult to interpret the effects of ectomycorrhizal fungi on *Pinus* and *Picea* growth, survival and resistance to biotic and abiotic stress. Therefore, the analysis did not provide concrete conclusions. However, the results of the analysis emphasize the importance of ectomycorrhizal fungi on conifer growth. The majority of the studies were conducted in growth facilities while very few were conducted in the field. There is need to conduct more experiments in the field to better understand the role of ectomycorrhizal fungi in conifer health and success. Also negative results must be published and a platform for this scenario must be created. I also urge researchers to report publication biases in their results. Therefore, there is still too sparse knowledge to provide recommendations on how ectomycorrhizal fungi can be used in forest management.

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## D-Biotic factor variables

The table shows variables used in the meta-analysis, data type and the calculated effect sizes

study_id	year	author	tree_genus	soil_type	ecm_genus	growth_conditions	stress_type	tree_age	tree_category	measured_parameter	data_type	effect_size	var_d	source
20	2021	Chu	pinus	composite	amanita	growth facility	insect	9 seedling	disease incidence	disease incidence	mean	-2.02780972	0.41398961	Hedges_d
20	2021	Chu	pinus	composite	suillus	growth facility	insect	9 seedling	disease incidence	disease incidence	mean	0.53158842	0.40463384	Hedges_d
211	1998	Manninen	pinus	media	cenococcum	growth facility	Insect	2.75 seedling	root length	root length	mean	-1.82440703	0.22571024	Hedges_d
211	1998	Manninen	pinus	media	cenococcum	growth facility	Insect	3.25 seedling	root length	root length	mean	-2.10582351	0.22887826	Hedges_d
218	1997	Gerhring	pinus	field	ecm	field	Insect	96 seedling	mortality	mortality	mean	-0.87014149	0.40580496	Hedges_d
218	1997	Gerhring	pinus	field	ecm	field	Insect	96 seedling	mortality	mortality	mean	-4.74396802	0.45626071	Hedges_d
277	2018	Piculell	pinus	field	ecm	growth facility	pathogen	5.5 seedling	relative growth rate	relative growth rate	mean	0.93802036	0.13500372	Hedges_d
277	2018	Piculell	pinus	field	ecm	growth facility	pathogen	5.5 seedling	relative growth rate	relative growth rate	mean	0	0.15123825	Hedges_d
277	2018	Piculell	pinus	field	ecm	growth facility	pathogen	5.5 seedling	relative growth rate	relative growth rate	mean	0.64744052	0.12515279	Hedges_d
277	2018	Piculell	pinus	field	ecm	growth facility	pathogen	5.5 seedling	relative growth rate	relative growth rate	mean	0	0.14031362	Hedges_d
323	2007	Liiri	pinus	composite	ecm	growth facility	insect	6.5 seedling	root length	root length	mean	0	0.13371318	Hedges_d
323	2007	Liiri	pinus	composite	ecm	growth facility	insect	6.5 seedling	root length	root length	mean	-0.49529781	0.15470792	Hedges_d
324	2007	Niemi	pinus	media	pisolithus	growth facility	pathogen	0.5 seedling	cell masses with developing embryo	cell masses with developing embryo	mean	0.94742786	0.47079149	Hedges_d
325	2006	Machon	pinus	composite	laccaria	greenhouse	pathogen	3.25 seedling	pre-emergence damping off	pre-emergence damping off	mean	0.58304539	0.46527513	Hedges_d
325	2006	Machon	pinus	composite	laccaria	greenhouse	pathogen	3.25 seedling	pre-emergence damping off	pre-emergence damping off	mean	-3.44056987	0.56827811	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	0.98923827	0.47158708	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	diameter	diameter	mean	0.3092284	0.25123464	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	root length	root length	mean	0.24324324	0.25122926	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	0.36978551	0.2512407	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	diameter	diameter	mean	-0.77815449	0.2513088	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	root length	root length	mean	0	0.25122054	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	11.3762552	0.26962635	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	diameter	diameter	mean	-0.09760033	0.25122195	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	root length	root length	mean	-0.17199895	0.2512249	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	12.3934138	0.27292727	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	diameter	diameter	mean	-0.07358928	0.25122134	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	root length	root length	mean	0.34399789	0.25123799	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	10.1199055	0.26589071	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	diameter	diameter	mean	-0.03771614	0.25122075	Hedges_d
326	2018	Suarez	pinus	composite	lactarius	growth facility	pathogen	5 seedling	root length	root length	mean	0	0.25122054	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	-1.02333836	0.25137488	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	diameter	diameter	mean	-0.38828141	0.25124277	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	root length	root length	mean	0	0.25122054	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	shoot height	shoot height	mean	-0.46028913	0.25125177	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	diameter	diameter	mean	-0.04689805	0.25122087	Hedges_d
326	2018	Suarez	pinus	composite	rhizopogon	growth facility	pathogen	5 seedling	root length	root length	mean	-0.34399789	0.25123799	Hedges_d
327	2020	Berk	pinus	field	wilcoxina	growth facility	pathogen	2.7 seedling	seedling height	seedling height	mean	-1.87664869	0.25173922	Hedges_d
329	2006	Mueller	pinus	composite	ecm	field	pathogen	96 mature tree	shoot growth	shoot growth	mean	-0.87347081	0.251333	Hedges_d
329	2006	Mueller	pinus	composite	ecm	field	pathogen	96 mature tree	mortality	mortality	mean	0	0.25122054	Hedges_d
334	1995	Hwang	pinus	composite	paxillus	growth facility	pathogen	2.5 seedling	seedling mortality	seedling mortality	mean	0.3355963	0.25123714	Hedges_d
334	1995	Hwang	pinus	composite	paxillus	growth facility	pathogen	2.5 seedling	seedling mortality	seedling mortality	mean	0.36323253	0.4632008	Hedges_d
334	1995	Hwang	pinus	composite	suillus	growth facility	pathogen	2.5 seedling	seedling mortality	seedling mortality	mean	0.4912867	0.30296563	Hedges_d
204	1999	Chakravarty	pinus	composite	paxillus	growth facility	pathogen	2 seedling	survival	survival	mean	-0.23141273	0.16487542	Hedges_d
204	1999	Chakravarty	pinus	composite	laccaria	growth facility	pathogen	2 seedling	survival	survival	mean	-0.19568249	0.16487517	Hedges_d
9	2022	Wang	pinus	thunbergia	hymenochaete	growth facility	pathogen	5 seedling	Infection rate	Infection rate	percentage	-1.82939167	0.85279501	logRR
9	2022	Wang	pinus	thunbergia	hymenochaete	growth facility	pathogen	5 seedling	Infection rate	Infection rate	percentage	0.001	0.85279501	logRR
25	2021	Lorenc	pinus	abies	ectovit	field	pathogen	na	Infection rate	Infection rate	percentage	0.09703581	0.69456387	logRR
25	2021	Lorenc	pinus	abies	ectovit	field	pathogen	na	Infection rate	Infection rate	percentage	-0.03783997	0.72120705	logRR
108	2010	Holusa	pinus	abies	ectovit	field	pathogen	72 mature tree	seedling height	seedling height	percentage	-0.25297888	0.2575645	logRR
108	2010	Holusa	pinus	abies	ectovit	field	pathogen	72 mature tree	root length	root length	percentage	-0.36050732	0.28872018	logRR
268	2019	Chu	pinus	tabulaeformis	suillus	growth facility	pathogen	0.9 seedling	cumulative mortality	cumulative mortality	percentage	-6.68461173	2.88602957	logRR
268	2019	Chu	pinus	tabulaeformis	suillus	growth facility	pathogen	0.9 seedling	cumulative mortality	cumulative mortality	percentage	-6.68461173	14.1368313	logRR
268	2019	Chu	pinus	tabulaeformis	schizophyllum	growth facility	pathogen	0.9 seedling	cumulative mortality	cumulative mortality	percentage	0.35468679	1.59326795	logRR
272	2001	Nieminen	pinus	silvestris	ecm	growth facility	insect	8.25 seedling	stem length	stem length	percentage	0.04481933	1.98817723	logRR
277	2018	Piculell	pinus	Taeda	thelephora	growth facility	pathogen	5.5 seedling	relative growth rate	relative growth rate	percentage	4.38507808	0.20500389	logRR
326	2018	Suarez	pinus	silvestris	lactarius	growth facility	pathogen	5 seedling	diameter	diameter	percentage	-0.03886452	0.36890751	logRR
326	2018	Suarez	pinus	silvestris	lactarius	growth facility	pathogen	5 seedling	root length	root length	percentage	-1.28519824	0.87050223	logRR
326	2018	Suarez	pinus	silvestris	lactarius	growth facility	pathogen	5 seedling	shoot height	shoot height	percentage	-1.28519824	0.87050223	logRR
334	1995	Hwang	pinus	banksiana	suillus	growth facility	pathogen	2.5 seedling	seedling mortality	seedling mortality	percentage	-0.32016753	0.22882448	logRR
336	2018	Velmala	pinus	abies	meliniomyces	growth facility	pathogen	12 seedling	shoot length	shoot length	percentage	0.11341758	0.18859114	logRR
336	2018	Velmala	pinus	abies	meliniomyces	growth facility	pathogen	12 seedling	shoot length	shoot length	percentage	-0.14939123	0.26261032	logRR
336	2018	Velmala	pinus	abies	meliniomyces	growth facility	pathogen	12 seedling	damaged needles	damaged needles	percentage	-0.58530065	0.38460264	logRR
336	2018	Velmala	pinus	abies	meliniomyces	growth facility	pathogen	12 seedling	damaged needles	damaged needles	percentage	-0.07855036	0.24306634	logRR
204	1999	Chakravarty	pinus	silvestris	citocybe	growth facility	pathogen	2 seedling	survival	survival	percentage	0.51082562	3.84177814	logRR
204	1999	Chakravarty	pinus	silvestris	paxillus	growth facility	pathogen	2 seedling	survival	survival	percentage	0.001	4.29685501	logRR
204	1999	Chakravarty	pinus	silvestris	laccaria	growth facility	pathogen	2 seedling	survival	survival	percentage	0.22314355	3.52766841	logRR
204	1999	Chakravarty	pinus	silvestris	citocybe	growth facility	pathogen	2 seedling	survival	survival	percentage	0.001	3.71931893	logRR
204	1999	Chakravarty	pinus	silvestris	paxillus	growth facility	pathogen	2 seedling	survival	survival	percentage	0.001	3.71931893	logRR
204	1999	Chakravarty	pinus	silvestris	laccaria	growth facility	pathogen	2 seedling	survival	survival	percentage	-0.28768207	4.01847585	logRR
204	1999	Chakravarty	pinus	silvestris	Citocybe	growth facility	pathogen	2 seedling	survival	survival	percentage	0.26436485	0.30795331	logRR
204	1999	Chakravarty	pinus	silvestris	paxillus	growth facility	pathogen	2 seedling	survival	survival	percentage	0.34281346	0.4782272	logRR
204	1999	Chakravarty	pinus	silvestris	laccaria	growth facility	pathogen	2 seedling	survival	survival	percentage	1.57242928	0.51657261	logRR
204	1999	Chakravarty	pinus	silvestris	Citocybe	growth facility	pathogen	2 seedling	survival	survival	percentage	0.56426201	0.19232944	logRR

## E -Included articles.

Author name	Year of publication	Title
Tapwal, A.	2022	Growth enhancement in containerized <i>Pinus gerardiana</i> seedlings inoculated with ectomycorrhizal fungi
McMahen, K.	2022	Soil microbial legacies influence plant survival and growth in mine reclamation
Fahey, C	2022	Effects of dual mycorrhizal inoculation on <i>Pinus strobus</i> seedlings are influenced by soil resource availability
Huang, L.-L.	2022	Ectomycorrhizal synthesis between two <i>Tuber</i> species and six tree species: are different host-fungus combinations having dissimilar impacts on host plant growth?
Wang, Y.	2022	Improvement of <i>Sphaeropsis</i> Shoot Blight Disease Resistance by Applying the Ectomycorrhizal Fungus <i>Hymenochaete</i> sp. R1 and Mycorrhizal Helper Bacterium <i>Bacillus pumilus</i> HR10 to <i>Pinus thunbergii</i>
Chen, H.	2022	Effects of <i>Suillus luteus</i> and <i>S. bovinus</i> on the physiological response and nutrient absorption of <i>Pinus massoniana</i> seedlings under phosphorus deficiency
Repac, I.	2022	Ectomycorrhiza-hydrogel additive enhanced growth of Norway spruce seedlings in a nutrient-poor peat substrate
Wang, J.	2021	Effects of ectomycorrhizal fungi ( <i>Suillus variegatus</i> ) on the growth, hydraulic function, and non-structural carbohydrates of <i>Pinus tabulaeformis</i> under drought stress
Castro, D.	2021	Effects of early, small-scale nitrogen addition on germination and early growth of scots pine ( <i>Pinus sylvestris</i> ) seedlings and on the recruitment of the root-associated fungal community
Madejon, P.	2021	Plant response to mycorrhizal inoculation and amendments on a contaminated soil
Chu, H.	2021	Inoculation With Ectomycorrhizal Fungi and Dark Septate Endophytes Contributes to the Resistance of <i>Pinus</i> spp. to Pine Wilt Disease
Taniguchi,	2021	Plantation soil inoculation combined with straw checkerboard barriers enhances ectomycorrhizal colonization and subsequent growth of nursery grown <i>Pinus tabulaeformis</i> seedlings in a dryland
Li, M.	2021	Role of <i>Suillus placidus</i> in improving the drought tolerance of masson pine ( <i>Pinus massoniana</i> lamb.) seedlings
Peng, L.	2021	Soil phosphorus mobilization and utilization by <i>Suillus</i> isolates and <i>Suillus</i> -mycorrhized pine plants
Lorenc,	2021	Influence of mycorrhizal preparation on seedling growth and <i>Armillaria</i> infestation
Liu, H.	2020	Identification of candidate genes conferring tolerance to aluminum stress in <i>Pinus massoniana</i> inoculated with ectomycorrhizal fungus
Gehring, C.	2020	Ectomycorrhizal and Dark Septate Fungal Associations of Pinyon Pine Are Differentially Affected by Experimental Drought and Warming
Verma, B.	2020	Biochar augmentation improves ectomycorrhizal colonisation, plant growth and soil fertility
Zhang, X.	2020	Colonization by <i>Tuber melanosporum</i> and <i>Tuber indicum</i> affects the growth of <i>Pinus armandii</i> and <i>phoD</i> alkaline phosphatase encoding bacterial community in the rhizosphere
Hewitt, R.E.	2020	Limited overall impacts of ectomycorrhizal inoculation on recruitment of boreal trees into Arctic tundra following wildfire belie species-specific responses
Li, X.	2020	Root-tip cutting and uniconazole treatment improve the colonization rate of <i>Tuber indicum</i> on <i>Pinus armandii</i> seedlings in the greenhouse
Gallart, M	2018	Host Genotype and Nitrogen Form Shape the Root Microbiome of <i>Pinus radiata</i>
Zhang, H.	2017	Prior contact of <i>Pinus tabulaeformis</i> with ectomycorrhizal fungi increases plant growth and survival from damping-off
Hazard, C.	2017	Strain identity of the ectomycorrhizal fungus <i>Laccaria bicolor</i> is more important than richness in regulating plant and fungal performance under nutrient rich conditions
Rudawska, M	2017	Forest litter amendment during nursery stage influence field performance and ectomycorrhizal community of Scots pine ( <i>Pinus sylvestris</i> L.) seedlings outplanted on four different sites
Hazard, C.	2017	Contrasting effects of intra- and interspecific identity and richness of ectomycorrhizal fungi on host plants, nutrient retention and multifunctionality
Nakashima, H.	2016	Effect of ectomycorrhizal composition on survival and growth of <i>Pinus thunbergii</i> seedlings varying in resistance to the pine wilt nematode
Yin, D.	2016	Synergistic effects between <i>Suillus luteus</i> and <i>Trichoderma virens</i> on growth of Korean spruce seedlings and drought resistance of Scotch pine seedlings
BarroetaveÁa, C	2016	Field performance of <i>Pinus ponderosa</i> seedlings inoculated with ectomycorrhizal fungi planted in steppe-grasslands of andean patagonia, Argentina

Vaario, L.-M.	2015	Variation among matsutake ectomycorrhizae in four clones of <i>Pinus sylvestris</i>
Franco, A.R.	2015	Effect of benfluralin on <i>Pinus pinea</i> seedlings mycorrhized with <i>Pisolithus tinctorius</i> and <i>Suillus bellinii</i> - Study of plant antioxidant response
Klavina, D.	2015	Seed provenance impacts growth and ectomycorrhizal colonisation of <i>Picea abies</i> seedlings
Repac •, I.	2015	Effects of substrate and ectomycorrhizal inoculation on the development of two-years-old container-grown Norway spruce ( <i>Picea abies</i> Karst.) seedlings
Sousa, N.R.	2014	A genotype dependent-response to cadmium contamination in soil is displayed by <i>Pinus pinaster</i> in symbiosis with different mycorrhizal fungi
Ito, Z.A.	2014	Influence of ectomycorrhizal inoculation on <i>Pinus wallichiana</i> and <i>Cedrus deodara</i> seedlings under nursery conditions
Onwuchekwa, N.E.	2014	Growth of mycorrhizal jack pine ( <i>Pinus banksiana</i> ) and white spruce ( <i>Picea glauca</i> ) seedlings planted in oil sands reclaimed areas
Dominguez, J.A.	2013	Short communication. Physiological effects of <i>Rhizopogon roseolus</i> on <i>Pinus halepensis</i> seedlings
Sanchez-Zabala, J.	2013	Physiological aspects underlying the improved outplanting performance of <i>Pinus pinaster</i> Ait. seedlings associated with ectomycorrhizal inoculation
Lazarevia, J..	2012	Mycorrhization of containerized <i>Pinus nigra</i> seedlings with <i>Suillus granulatus</i> under open field conditions
Ragonezi, C.	2012	<i>Pisolithus Arhizus</i> (Scop.) rauschert improves growth of adventitious roots and acclimatization of In vitro regenerated plantlets of <i>Pinus pinea</i> L.
Sousa, N.R.	2012	Mycorrhizal symbiosis affected by different genotypes of <i>Pinus pinaster</i>
Otgonsuren, B.	2012	<i>Pinus sylvestris</i> can form ectomycorrhiza with <i>Phialocephala fortinii</i>
Dominguez, J.A.	2012	The combined effects of <i>Pseudomonas fluorescens</i> and <i>Tuber melanosporum</i> on the quality of <i>Pinus halepensis</i> seedlings
Oliveira, R.S.	2012	Combined use of <i>Pinus pinaster</i> plus and inoculation with selected ectomycorrhizal fungi as an ecotechnology to improve plant performance
Sousa, N.R.	2012	Ectomycorrhizal fungi as an alternative to the use of chemical fertilisers in nursery production of <i>Pinus pinaster</i>
Sousa, N.R.	2012	The effect of ectomycorrhizal fungi forming symbiosis with <i>Pinus pinaster</i> seedlings exposed to cadmium
Sousa, N.R.	2011	Reforestation of burned stands: The effect of ectomycorrhizal fungi on <i>Pinus pinaster</i> establishment
Buscardo, E.	2011	Common environmental factors explain both ectomycorrhizal species diversity and pine regeneration variability in a post-fire Mediterranean forest
Wagg, C.	2011	Soil microbial communities from an elevational cline differ in their effect on conifer seedling growth
Koele, N.	2011	Differences in growth and nutrient uptake from a coarse-soil substrate by ectomycorrhizal- and fungicide-treated <i>Picea abies</i> seedlings
Repac, I.	2011	Testing of microbial additives in the rooting of Norway spruce ( <i>Picea abies</i> [L.] Karst.) stem cuttings
Sarjala, T.	2010	Mycorrhiza formation is not needed for early growth induction and growth-related changes in polyamines in Scots pine seedlings in vitro
Vaario, L.-M	2010	Ectomycorrhization of <i>Tricholoma matsutake</i> and two major conifers in Finland-an assessment of in vitro mycorrhiza formation
Leski, T.	2010	Ectomycorrhizal community structure of different genotypes of Scots pine under forest nursery conditions
Holusa, J.	2009	Impact of mycorrhizal inoculation on spruce seedling: Comparisons of a 5-year experiment in forests infested by honey fungus
Karst, J.	2009	Ectomycorrhizal colonization and intraspecific variation in growth responses of lodgepole pine
CorrÁ, A.	2008	Response of plants to ectomycorrhizae in N-limited conditions: Which factors determine its variation?
Zhu, J.-J	2008	The role of ectomycorrhizal fungi in alleviating pine decline in semiarid sandy soil of northern China: An experimental approach
Jha, B.N.	2008	Effect of ectomycorrhizal development on growth in pine seedlings
Repac •, I.	2007	Ectomycorrhiza formation and growth of <i>Picea abies</i> seedlings inoculated with alginate-bead fungal inoculum in peat and bark compost substrates
Rincolin, A.	2007	Inoculation of <i>Pinus halepensis</i> Mill. with selected ectomycorrhizal fungi improves seedling establishment 2 years after planting in a degraded gypsum soil
KozdrÁj, J.	2007	Mycorrhizal fungi and ectomycorrhiza associated bacteria isolated from an industrial desert soil protect pine seedlings against Cd(II) impact
Dominguez-Nunez, JA	2013	Effects of <i>Pseudomonas fluorescens</i> on the Water Parameters of Mycorrhizal and Non-Mycorrhizal Seedlings of <i>Pinus halepensis</i>

Probanza, A	2001	Effects of inoculation with PGPR <i>Bacillus</i> and <i>Pisolithus tinctorius</i> on <i>Pinus pinea</i> L. growth, bacterial rhizosphere colonization, and mycorrhizal infection
Rincon, A.	2005	Effects of ectomycorrhizal inoculation and the type of substrate on mycorrhization, growth and nutrition of containerised <i>Pinus pinea</i> L. seedlings produced in a commercial nursery
Choi, D.S.	2005	Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of <i>Pinus densiflora</i> seedlings grown under elevated CO <sub>2</sub> concentrations
Kim, C.-G. and Power, S.A. and Bell, J.N.B.	2004	Response of <i>Pinus sylvestris</i> seedlings to cadmium and mycorrhizal colonisation
Dunabeitia, M.K.	2004	Differential responses of three fungal species to environmental factors and their role in the mycorrhization of <i>Pinus radiata</i> D. Don
Mari, S.	2003	Genetic variation in nitrogen uptake and growth in mycorrhizal and nonmycorrhizal <i>Picea abies</i> (L.) karst. seedlings
van Scholl.	2005	Effect of ectomycorrhizal colonization on the uptake of Ca, Mg and Al by <i>Pinus sylvestris</i> under aluminium toxicity
Guerin-Laguet.	2003	The ectomycorrhizal symbiosis between <i>Lactarius deliciosus</i> and <i>Pinus sylvestris</i> in forest soil samples: Symbiotic efficiency and development on roots of a rDNA internal transcribed spacer-selected isolate of <i>L. deliciosus</i>
Ahonen-Jonnarth,	2001	Effects of elevated nickel and cadmium concentrations on growth and nutrient uptake of mycorrhizal and non-mycorrhizal <i>Pinus sylvestris</i> seedlings
Hartley, J.	1999	The effects of multiple metal contamination on ectomycorrhizal Scots pine ( <i>Pinus sylvestris</i> ) seedlings
Chakravarty, P.	1999	Integrated control of <i>Fusarium</i> damping-off in conifer seedlings
Hartley, J.	1999	Cross-colonization of scots pine ( <i>Pinus sylvestris</i> ) seedlings by the ectomycorrhizal fungus <i>Paxillus involutus</i> in the presence of inhibitory levels of Cd and Zn
Cram, M.M.	1999	Successful reforestation of south carolina sandhills is not influenced by seedling inoculation with <i>Pisolithus tinctorius</i> in the nursery
Manninen, A.-M.	1998	Susceptibility of ectomycorrhizal and nonmycorrhizal Scots pine ( <i>Pinus sylvestris</i> ) seedlings to a generalist insect herbivore, <i>Lygus rugulipennis</i> , at two nitrogen availability levels
Scagel, C.F.	1998	Relationships between differential in vitro Indole-Acetic Acid or ethylene production capacity by ectomycorrhizal fungi and conifer seedling responses in symbiosis
Scagel, C.F.	1998	Influence of ectomycorrhizal fungal inoculation on growth and root IAA concentrations of transplanted conifers
Gehring, C.A.	1997	Three-way interactions among ectomycorrhizal mutualists, scale insects, and resistant and susceptible pinyon pines
Shaw, T.M.	1995	Interactions between ectomycorrhizal and saprotrophic fungi on agar and in association with seedlings of lodgepole pine ( <i>Pinus contorta</i> )
BONELLO, P.	1993	Ozone effects on root • disease susceptibility and defence responses in mycorrhizal and non • mycorrhizal seedlings of Scots pine ( <i>Pinus sylvestris</i> L.)
WILKINS, D.A.	1989	The effects of aluminium and <i>Paxillus involutus</i> Fr. on the growth of Norway spruce [ <i>Picea abies</i> (L.) Karst.]
Berry, C.R.	1977	Growth of loblolly pine seedlings in strip mined kaolin spoil as influenced by sewage sludge
Chu, H.L.	2019	The Dark Septate Endophytes and Ectomycorrhizal Fungi Effect on <i>Pinus tabulaeformis</i> Carr. Seedling Growth and their Potential Effects to Pine Wilt Disease Resistance
Nadeau, M.B.	2018	Mycorrhizae and Rhizobacteria on Precambrian Rocky Gold Mine Tailings: I. Mine-Adapted Symbionts Promote White Spruce Health and Growth
Nowak, J.	2006	Loblolly pine and slash pine responses to acute aluminum and acid exposures
Nieminen, J.K.	2001	Influence of carbon and nutrient additions on a decomposer food chain and the growth of pine seedlings in microcosms
Piculell, B.J.	2018	Genetically determined fungal pathogen tolerance and soil variation influence ectomycorrhizal traits of loblolly pine

Menkis, A.	2007	Afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi-impact on plant performance and ectomycorrhizal community
Jenkins, M.L.	2018	Scorched Earth: <i>Suillus</i> colonization of <i>Pinus albicaulis</i> seedlings planted in wildfire-impacted soil affects seedling biomass, foliar nutrient content, and isotope signatures
Kayama, M .	2006	Comparison of growth characteristics and tolerance to serpentine soil of three ectomycorrhizal spruce seedlings in northern Japan
SVENSON, S.E.	1991	Ectomycorrhizae and drought acclimation influence water relations and growth of loblolly-pine
Wen, Z.G.	2019	Distributions and Compositions of Brominated Diphenyl Ethers-209 in Pine Seedlings Inoculated with Ectomycorrhizal Fungi
Sun, Y.J .	2020	Effects of slippery jack ( <i>Suillus luteus</i> ) on the heavy metal accumulation and soil properties of masson's pine ( <i>Pinus massoniana</i> Lamb) in a mining area of China
Velmala, S.M.	2018	Ectomycorrhizal fungi increase the vitality of Norway spruce seedlings under the pressure of <i>Heterobasidion</i> root rot in vitro but may increase susceptibility to foliar necrotrophs
Niemi, K.	2007	Spermidine and the ectomycorrhizal fungus <i>Pisolithus tinctorius</i> synergistically induce maturation of Scots pine embryogenic cultures
Hwang, S.F.	1995	The effect of two ectomycorrhizal fungi, <i>Paxillus involutus</i> and <i>Suillus tomentosus</i> , and of <i>Bacillus subtilis</i> on <i>Fusarium</i> damping-off in jack pine seedlings
Wang, Y.H.	2022	Mycorrhiza helper bacterium <i>Bacillus pumilus</i> HR10 improves growth and nutritional status of <i>Pinus thunbergii</i> by promoting mycorrhizal proliferation
Mueller, R.C.	2006	Interactions between an above-ground plant parasite and below-ground ectomycorrhizal fungal communities on pinyon pine
Beck, J.L.	2020	Changes in soil fungal communities following anthropogenic disturbance are linked to decreased lodgepole pine seedling performance
Suarez, J.O.	2018	Effects of <i>Lactarius deliciosus</i> and <i>Rhizopogon roseolus</i> ectomycorrhizal fungi on seeds and seedlings of Scots and stone pines inoculated with <i>Fusarium oxysporum</i> and <i>Fusarium verticillioides</i>
Machon, P.	2006	Influence of the ectomycorrhizal fungus <i>Laccaria laccata</i> on pre-emergence, post-emergence and late damping-off by <i>Fusarium moniliforme</i> and <i>F. oxysporum</i> on Scots pine seedlings
Niemi, K	2007	<i>Suillus variegatus</i> causes significant changes in the content of individual polyamines and flavonoids in Scots pine seedlings during mycorrhiza formation in vitro
Liiri, M	2007	Variable impacts of enchytraeid worms and ectomycorrhizal fungi on plant growth in raw humus soil treated with wood ash
Yin, DC	2018	Ectomycorrhizal fungus enhances drought tolerance of <i>Pinus sylvestris</i> var. <i>mongolica</i> seedlings and improves soil condition
Niemi, K .	2002	<i>Pisolithus tinctorius</i> promotes germination and forms mycorrhizal structures in Scots pine somatic embryos in vitro
Hartley-Whitaker, J.	2000	Sensitivity to Cd or Zn of host and symbiont of ectomycorrhizal <i>Pinus sylvestris</i> L. (Scots pine) seedlings
van Hees,	2006	The biogeochemical impact of ectomycorrhizal conifers on major soil elements (Al, Fe, K and Si)
Kipfer, T.	2015	Drought resistance of <i>Pinus sylvestris</i> seedlings conferred by plastic root architecture rather than ectomycorrhizal colonisation
Dominguez Nunez, J.A.	2008	The effect of Tuber melanosporum Vitt. mycorrhization on growth, nutrition, and water relations of <i>Quercus petraea</i> Liebl., <i>Quercus faginea</i> Lamk., and <i>Pinus halepensis</i> Mill. seedlings
Nadeau, M.B.	2018	Mycorrhizae and Rhizobacteria on Precambrian Rocky Gold Mine Tailings: II. Mine-Adapted Symbionts Alleviate Soil Element Imbalance for a Better Nutritional Status of White Spruce Seedlings
Franco, A.R.	2015	Inoculation of <i>Pinus pinea</i> seedlings with <i>Pisolithus tinctorius</i> and <i>Suillus bellinii</i> promotes plant growth in benfluralin contaminated soil
Mueller, R.C.	2019	Legacy effects of tree mortality mediated by ectomycorrhizal fungal communities
Menkis, A.	2011	Mycorrhization, Establishment and Growth of Outplanted <i>Picea abies</i> Seedlings Produced under Different Cultivation Systems

Kim, C.G.	2004	Effects of host plant exposure to cadmium on mycorrhizal infection and soluble carbohydrate levels of <i>Pinus sylvestris</i> seedlings
Tahara, C.	2005	Ectomycorrhizal association enhances Al tolerance by inducing citrate secretion in <i>Pinus densiflora</i>
Patterson, A.	2019	Common garden experiments disentangle plant genetic and environmental contributions to ectomycorrhizal fungal community structure





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