# What are the impacts of mixed forest stands on forest pathogens? A systematic review protocol

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## Abstract

### Background

The impacts of forest pathogens on tree health are on the increase as a result of increasing trade globalisation and anthropogenically driven environmental change. Diversifying planted forests to reduce the risks associated with large scale disturbances, such pathogen outbreaks, is a major theme in sustainable forest management. However, practitioners currently lack evidence for the effectiveness of this approach that could help them to decide whether or not to implement this forest management strategy.

### Methods

This systematic review will take a two-pronged approach to assimilating the available evidence on the effectiveness of using mixed species stands to control pathogen damage. In the first instance, we will provide a narrative synthesis of the results from previous studies, and suggest potential mechanistic explanations for these results. In the second instance, if sufficient quantitative data is available, we will use meta-analysis to quantify the overall relationship between forest diversity and pathogen damage, and explore how this relationship is affected by factors such as pathogen specialisation and forest type. Search strategy will include the use of both bibliographic databases and internet searches and the review will also synthesise unpublished data sets contributed by stakeholders, following critical appraisal of all data sources.

## Background

The area of planted forests around the globe is on the increase (Payn et al., 2015). Over 99.9% of these are planted as monocultures (Nichols et al., 2006), even though several decadal-long studies have shown that more diverse plantations can show higher levels of productivity (Bielak et al., 2014; Pretzsch et al., 2013), and other ecosystem functions (Nadrowski et al., 2010). Diversifying stands is one of the key principles underlying sustainable forestry approaches such as "Continuous Cover Forestry" and "Close-to-Nature Silviculture" (Brang et al., 2014; Mason et al., 1999). Planting stands of multiple species is also often promoted as a method of economic "bet-hedging" for foresters, reducing the risk associated with large scale disturbances such as the impact of emerging pests and pathogens (Jactel et al., 2009). If one species is removed due to the impact of a pest organism, the economic value of the stand is not totally lost.

In addition to economic resilience, it is also often assumed that mixed forests are intrinsically more resistant to pathogen damage compared to monocultures, as can be the case in agricultural systems (Mundt, 2002) and experimental grassland communities (Mitchell et al., 2002). A more species rich planting may reduce pathogen damage via the dilution effect, which describes a reduction in susceptible host density leading to reduced colonisation of pathogens by hosts (Keesing et al., 2010). However, the lack of clear empirical evidence for the dilution effect on forest pathogens has been noted by previous authors (Koricheva et al., 2006; Pautasso et al. , 2005). A recent broad scale meta-analysis of the dilution effect across several study systems included just one article from forests (Civitello et al., 2015). Whether this represents a real knowledge gap, or simply a lack of dissemination of relevant studies, remains unclear. There are innate difficulties involved in carrying out long term experimental studies on long-lived, large woody organisms compared to short-lived agricultural crops and grassland species, the latter of which dominate the popular diversity-disease literature (Mitchell et al., 2002; Parker et al., 2015). However, these same difficulties may result in differences in the response of mixed forest stands to pathogens compared to other ecosystems. In particular, Mundt (2002) notes that the larger size of woody species compared to crop species can lead to increased frequency of autoinfection, reducing the effectiveness of using mixtures to lower pathogen damage. Furthermore, given the long age of most forest plantations, the durability of a mixed stand to disease progression over the entire length of the rotation has to be questioned.

In contrast to pathogens, the effects of forest stand diversity on insect pests have been a topic of greater study. Major theories concerning the effects of plant diversification on insect herbivory are shown in table 1. Recently, Jactel et al (2017) proposed expanding the use of the terms associational resistance (a reduction in pest damage in more diverse stands) and associational susceptibility (an increase in pest damage in more diverse stands) to all biotic agents of damage in plant communities, including both pests and pathogens. Several meta-analyses have shown that associational resistance, rather than associational susceptibility, is the most common response for insect herbivores (Castagneyrol et al., 2014; Jactel & Brockerhoff, 2007; Vehviläinen et al., 2007). These studies have also revealed that a key driver of the direction of associational effects in mixed forests is the degree of herbivore specialisation. In a study of seven long term forest experiments, Vehviläinen et al (2007) found that damage by specialist herbivores (leaf miners) was consistently reduced in more diverse stands. Jactel and Brockerhoeff (2007), in a meta-analysis of literature from 1966-2006, also found that damage by specialist herbivores was consistently reduced in mixed stands, while the response of generalist insects was more variable. Castagneyrol et al (2014) included phylogenetic diversity as a moderator in the meta-analysis, and showed that while specialist herbivores were consistently reduced in mixed stands, generalist herbivores were only reduced in the presence of highly unrelated hosts (in mixtures of broadleaved and coniferous species). Thus, the palatability of alternative hosts for generalist herbivores decreased with increasing phylogenetic diversity from the focal host species. Because the most damaging herbivores are normally specialist species, this may explain the overall tendency towards associational resistance in mixed stands, rather than associational susceptibility (Jactel & Brockerhoff, 2007; although see Kambach et al 2016).

In contrast to insect herbivores, the authors are not aware of any systematic synthesis of the literature concerning mixed stands and forest pathogens. As a result, both the overall *response* (whether tree diversity increases or decreases susceptibility to pathogen outbreak) and the *mechanisms* that could explain this response remain unknown. Factors such as pathogen specialisation, virulence level and dispersal method may all play a role in determining whether forest diversity increases or reduces resistance to pathogens. For instance, pathogens that are dependent on insect vectors for dispersal may show responses in mixed forests akin to that for insect herbivores, while the response of passive wind-dispersed pathogens may depend on factors such as physical or chemical apparency of hosts in mixed stands (Castagneyrol et al., 2013) and the dispersal kernel of the pathogen (Mundt, 2002). Studies of conifer plantations suggest that for root rot pathogens such as *Armillaria* sp., the requirement for root-to-root contact between susceptible host species in order for transmission to take place could lead to associational resistance in mixed stands (Gerlach et al., 1997). In addition to direct interference with pathogen dispersal, planting mixed stands of tree species may alter the soil microbial community, increasing the abundance of antagonistic fungi that could outcompete pathogenic species (Fedorov & Poleschuk, 1981).

The need for management measures to control emerging forest diseases has never been more prescient

| Theory | Description | Representative references |
|---|---|---|
| Associational Resistance (Tahvanainen & Root, 1972) | Increased plant diversity reduces pest damage as the presence of non-susceptible neighbouring hosts interferes with physical and / or chemical cues used by pest to find host | Forests: (Jactel & Brockerhoff, 2007)<br><br>Grassland: (Hambäck, Ågren, & Ericson, 2000) |
| Associational Susceptibility (Brown & Ewel, 1987) | Increased plant diversity increases pest damage, as pest spillover occurs from other susceptible hosts | Forests: (Castagneyrol, Lagache, Giffard, Kremer, & Jactel, 2012)<br><br>Grassland: (Utsumi, Ando, Craig, & Ohgushi, 2011) |
| Natural Enemies Hypothesis (Elton, 1958) | More diverse plant assemblages have greater diversity and abundance of natural enemies of pests, leading to reduced pest damage overall | Forests: (Sobek, Scherber, Steffan-Dewenter, & Tscharntke, 2009)<br><br>Grassland: (Johnson, Lajeunesse, & Agrawal, 2006) |

**Table 1:** The three major theories describing the effects of plant diversification on pest organisms.

(Pautasso et al., 2015). Highly virulent, invasive pathogens represent a major threat to forest production and biodiversity in an era of global change (Freer-Smith & Webber, 2015). Increasing trade globalisation poses major plant health biosecurity risks, and has already led to the spread of highly virulent pathogens such as Ash Dieback (*Hymenoscyphus fraxineus),* causing major mortality of European ash, *Fraxinus excelsior* (Pautasso et al., 2013). Also in Europe, emerging highly virulent *Phytophthora* species threaten economically conifer species such as juniper and larch (Brasier & Jung, 2006). Climate change could also lead to increases in the frequency and severity of plant pathogen outbreaks (Ramsfield et al., 2016). Although breeding for genetic resistance to diseases such as Ash Dieback is underway, this is a long-term solution and may be threatened by the rapid co-evolution of pathogens to overcome host resistance (McKinney et al., 2014). Thus, there is a need for durable strategies to reduce infection transmission and improve overall forest productivity and resilience to disease (Ennos, 2015). A shift away from planting single-aged stands of a single species, towards planting mixtures of species, is often promoted as one such strategy. However, the evidence its effectiveness and generality has yet to be synthesised in a systematic review.

## Engagement with Stakeholders

Engagement with stakeholders who work on mixed forests from several organisations was sought early on in the conception of the study. Several experts confirmed the relevance and potential impact of the systematic review. Stakeholders from the international TreeDivNet network of experimental forestry plantations have also provided advice in study conception and meta-analytic approaches, and contributed papers to the test library used in the scoping searches. Conversations with stakeholders were used to formulate secondary questions and produce the list of potential moderators provided in this protocol. Moreover, TreeDivNet members contributed unpublished data sets for potential incorporation into the study (described below). A list of further stakeholders have been contacted to request knowledge of additional sources of studies and/or unpublished data for contribution to the review.

Objectives of the Review

This review aims to synthesise the existing evidence concerning mixed forest stands and pathogen outbreak. We aim to incorporate both older studies in forest plantations (e.g. Gerlach et al., 1997; Peri, Korhonen, & Sairanen, 1990) and more recent studies from young experimental forests (e.g. Hantsch et al., 2014; Dillen, Verheyen, & Smit, 2016). In addition, using quantitative synthesis (meta-analysis), we aim to explore possible sources of heterogeneity in results between studies.

**PECO – Components of the Primary Question**

***Primary Question:*** *What are the impacts of mixed forest stands on forest pathogens?*

*Population*

Trees, defined as large woody organisms.

*Exposure*

Presence of mixtures of tree **species** in a forest stand

*Comparator (control)*

Pure stands (stands of only one tree species)

*Outcome*

Measures of pathogen infection on trees, including:
- tree level metrics of damage (including: number or proportion of affected leaves/branches per tree, % leaf damage by pathogen, lesion or canker size or number, % crown dieback)
- stand level mortality (proportion or number of affected trees per plot or stand)
- incidence rate (% infected trees per plot or stand)

**Secondary questions**

We also aim to explore sources of heterogeneity in response, both through qualitative synthesis in a narrative review, and, if enough quantitative data is available, using mixed effect meta-analysis models. In particular, we aim to address the following questions:
- Does the response of pathogens to tree species mixtures depend on the degree of host dilution?
- Does the reponse of pathogens to tree species mixtures depend on pathogen specialisation?
- Does the response of pathogens to tree species mixtures differ in stands where trees are of the same type versus different type (i.e. pure broadleaved or conifer versus mixed)?
- Are there differences in pathogen response dependent on pathogen group (fungal, viral, bacterial)?
- Are there differences in pathogen response dependent on the organ where damage occurs (e.g. foliar pathogens, root pathogens or vascular pathogens?)

Search strategy

There are four potential sources of studies to be included in the systematic review, shown in figure 1.

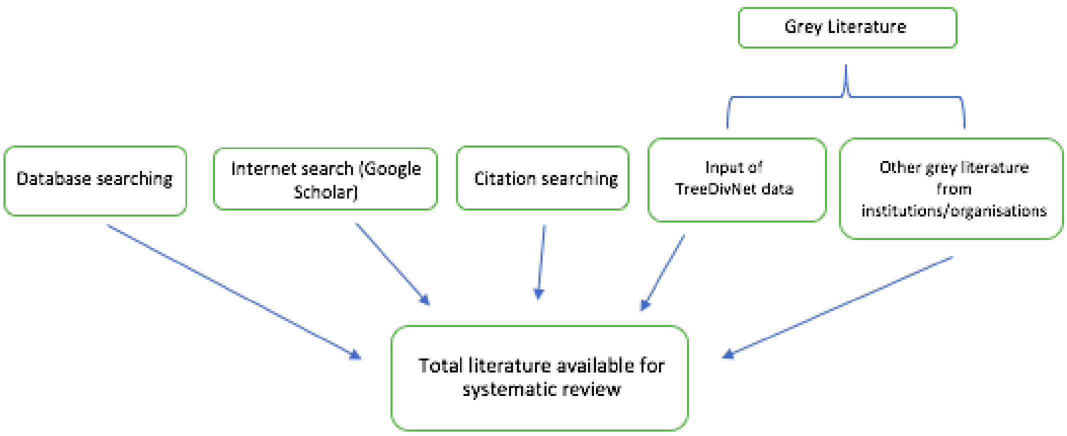


**Figure 1:** Sources of studies for inclusion in the systematic review.

**Bibliographic Databases**

The following 3 bibliographic databases will be used to source studies for the systematic review:

- Scopus: (http://www.scopus.com)
- Thomson Reuters Web of Science: (https://www.webofknowledge.com)
- CAB Abstracts: (http://www.ovid.com/site/catalog/databases/31.jsp)

**Search terms**

A scoping study conducted using the database Scopus was used to identify the search terms for use in bibliographic databases and internet searches. This was done by testing search strings in Scopus using the Field Tag (TITLE-ABS-KEY) to search for relevant titles, abstracts and keywords. Search terms were sourced from previous meta-analyses and systematic reviews on themes of mixed forests and/or insect herbivory and forest pathogens, and from the keywords of studies identified *a priori* as highly relevant (see Appendix I). Search results were ordered based on relevance, and limited to studies in the agriculture and biological sciences and environmental sciences topics to remove results from non-relevant subjects. For each search combination, the total number of hits and the number of relevant papers (assessed based on title) in the first 200 hits was recorded. In addition, each search combination was scrutinised for its ability to retrieve a list of **12** studies identified a priori as highly relevant to the primary question (test library, Appendix I). These studies were sourced from the personal libraries of the authors and stakeholders. Although all contain information relevant to the primary question, they utilise a range of observational and experimental study systems and the phrasing related to the primary question differs in each case; not all combinations of search terms were able to retrieve all of these papers. The final search terms were able to retrieve all of these papers, and achieved a good balance between relevant and non-relevant studies in the first 200 results.

The final search terms and their relevance to each of the PECO question elements is summarised in Table 2. Appendix III shows the exact search terms to be used in each database, the number of hits returned when initially searched, and search date. The large discrepancy in numbers of hits returned from Scopus compared to Web of Science and CABI is likely to be due to the restricted field tag used (TITLE-ABS-KEY) combined with differences in the literature available from Scopus, as without use of this field tag the number of hits returned by this database are typically extremely high (a comparison search using the same search terms without this field tag in Scopus returned over 37,000 hits).

Depending on the precise nature of the database in question and its capacity to deal with Boolean operators, the structure of search strings may vary. This will be recorded in each case along with the date when each database was searched.

Due to the large number of search results from irrelevant topics, the results of the Scopus search will be limited to journals in the “Agricultural and Biological Sciences” and “Environmental Science” categories. In Web of Science, results of searches will be limited to the following research areas in order to narrow the search: Agriculture OR Forestry OR Environmental Sciences OR Ecology OR Plant sciences.

Search results from all bibliographic and internet searches will be exported to reference manager software (EndNote) before further screening.

| PECO question component | Terms | Search terms |
|---|---|---|
| Population | Forest | tree* OR forest* OR plantation OR wood* OR orchard OR coppice OR shrub OR abies OR acer OR betula OR eucalyptus OR fagus OR fraxinus OR picea OR pinus OR populus OR quercus |
| Exposure | Mixed Stands | "mixed species" OR "mixed-species" OR "mixed stand*" OR "tree divers*" OR "tree species divers*" OR "host species divers*" OR "host divers*" OR "community divers*" OR “forest divers*” OR "forest species divers*" OR "divers* stand" OR "divers* plant*" OR "divers* forest" OR "divers* wood*" OR "tree species rich*" OR "host species rich*" OR "forest species rich*" OR polyculture* OR "landscape heterogen*" OR "heterogen* landscape" OR "species mix*" OR "diversity-disease" OR "species composition" |
| Comparator | Forest Pathogen Damage | pathogen OR disease OR infection OR "root rot" OR "butt rot" OR fung* OR lesion OR canker OR bacteria* OR viral OR virus OR Phytophthora OR Armillaria OR Heterobasidion |

**Table 2:** List of search strings to be used in bibliographic databases and their relation to each component of the primary question. Each section is combined using ‘AND’ (for exact search strings with Boolean operators for each search engine, see Appendix III).

### Internet Searches

To increase the comprehensiveness of the search strategy, internet searches using Google Scholar (http://scholar.google.com) will also be carried out. Haddaway et al (2015) showed only poor overlap between results from Web of Science and Google Scholar when similar search strings were used, thus, the additional Google Scholar searches are likely to increase the pool of potentially relevant studies available for systematic review. The same search terms will be utilised in Google Scholar as for the bibliographic database searches, with modifications as Boolean search operators cannot be used in the Google search engine. Details of the precise search terms used, number of hits, and the dates searched will be recorded. The first 1000 articles retrieved from Google Scholar will be downloaded and citation data extracted using web scraping tools, before article screening begins.

**Citation searching**

The references of each paper found to be relevant at the full text level will also be scrutinised for further relevant papers for screening.

**Personal libraries of contributors**

The personal libraries of the authors will be searched for sources of additional reference material, and stakeholders, where appropriate, will also be asked for sources of relevant reference material for screening.

**Grey Literature**

The review may also include an input of relevant grey literature (non peer reviewed literature), from the following sources:

***Input of unpublished data from tree diversity experiments:***

The review will synthesise a set of as yet unpublished data sets collected from TreeDivNet, the experimental long term forest diversity network (http://www.treedivnet.ugent.be, see Verheyen et al., 2016). Before inclusion in the study, this data will be subject to the same critical appraisal outlined below, utilising metadata submitted by each contributor (see Appendix II).

***Input of data from other stakeholders:***

Enquiries will be made through contacts at the following organisations, institutions and working groups as to the addition of further data, grey literature and/or relevant references for the systematic review:

Centre for International Forest Research (CIFOR)
EU COST action on Mixed Forests (EUMIXFOR)
EUFORGEN
European Forest Institute
Food and Agriculture Organisation of the United Nations
Forest Europe
Forest Research (UK)
French National Institute for Agricultural Research (INRA)
International Union of Forestry Research Organisations (IUFRO)
United States Department of Agriculture
World Agroforestry Centre

Contributors will be asked to submit all data files along with a completed meta data form (see Appendix II).

In all cases, studies from grey literature will undergo the same screening process as for peer-reviewed literature, and will be subject to the same process of critical appraisal before inclusion in the review.

Article Screening and Study Inclusion Criteria

**Article Screening**

Following the removal of duplicates, studies will be assessed for their relevance for inclusion in a step wise process: first on title, then on title and abstract, then on full text.

Screening at both the title level and abstract level will be completed using the online tool Abstrackr: (http://abstrackr.cebm.brown.edu/ ).

**Consistency Checking during Screening**

Screening for inclusion will be undertaken by the same reviewer (EF). In addition, prior to the start of each stage of the screening process, a second reviewer will screen a sub-set of articles: 200 articles at the title stage, and a smaller number of articles at the title and abstract (25 or 10%, whichever number is larger) and full text (10 or 10%, whichever number is larger) screening stages, to check for consistency in the study exclusion process. A kappa test will be performed (CEE, 2013) to check for consistency, with a kappa value > 0.6 indicating that reviewers are consistent in their assessments of study relevance. If k < 0.6, then reviewers must discuss the possible sources of inconsistency and re-assess the requirements for study inclusion. The screening process will then be repeated and re-tested until k > 0.6.

**Study Inclusion Criteria**

For inclusion in the study, the full text must include:

1) *Relevant population:* Tree species. The review includes studies of timber crops (long rotation and short rotation species such as poplar and willow), fruit trees and other tree crops.
2) *Relevant exposure and comparison:* A comparison between single-species stands and mixed-species stands. Comparisons between stands must be made at the same time (within the same year / season) and within the same geographical region. The comparison must be made where stands actually differ in their diversity at the time of the study, i.e., not studies where tree species mixtures were used only at the establishment stage prior to thinning. Such studies effectively contain more than one treatment (both use of tree species mixtures, and thinning leading to lower plantation density) which are confounded, thus they should not be included in the review. The review includes both ‘observational’ studies (defined here as unmanaged or semi-natural woodlands) and ‘experimental’ studies (defined as any planted forests where mixtures are compared to monocultures). The review does not include studies of trees outside of forested areas (such as hedgerow or roadside trees).
3) *Relevant outcome:* Information on damage, incidence rate or mortality by tree pathogens in stands differing in diversity. If the study is a primary paper (rather than a review or meta-analysis), it must include some quantitative assessment of the degree of pathogen damage, i.e. not simply presence/absence data (e.g. “presence” in pure stands versus “absence” in mixed stands: data must be present at a higher spatial resolution than this such as at the individual tree level). It is possible that where studies have measured several aspects of the growth of mixed and pure stands, exposure to pathogens may have been measured, but no evidence for pathogen damage has been found in either type of stand. In this case, these studies are not relevant to the primary question as it is likely that they remain unchallenged by pathogens. Because neither type of stand shows pathogen outbreak, it is likely that differences in planting design are not the reason for lack of pathogen exposure. This is not the same as studies showing no difference in pathogen damage between mixed and pure stands: such studies would be included as they are highly relevant to primary question of the review.

The review is limited to studies where an English language version is available. Studies will be considered for inclusion in the systematic review dated anywhere from the start of the database references - present, although the scoping study in Scopus suggested very few relevant papers will be retrieved from earlier than 1950. At this time there are no geographical restrictions on studies to be included in the review.

At each stage of the screening process, the number of articles that are excluded will be recorded. Reference caches containing the articles that have been excluded at each stage will be retained. Where studies are rejected at the full text stage, the reasons for rejection will also be recorded. A list of articles that have been rejected at the full text stage will be submitted (along with the reasons for exclusion) as part of the supplementary material along with the systematic review.

Critical Appraisal

After studies deemed to be relevant at full text level have been assimilated, a critical appraisal of study validity will take place. Studies will be split into three groups: (1) low risk of bias; (2) medium risk of bias; (3) high risk of bias. Although the same reviewer (EF) will carry out the majority of the study quality assessment a second reviewer will screen a randomised subset of studies (10% or 10 studies, whichever number is largest) to check for consistency and ensure repeatability of the critical appraisal process. The critical appraisal process focuses on internal validity (risk of bias) of studies, rather than external validity (relevance to the review), as there are no geographical or temporal limitations on the systematic review.

Studies will be scored to ascertain risk of bias based on the following categories:

1) **Presence of treatment replication:** use of blocking to account for spatial variability OR presence of multiple study sites: Both (2) / Either (1) / Neither (0)
2) **Degree of replication:** following from point 1, the degree of replication of each treatment/diversity level: 3 or more replicates (2) / two replicates (1) / no replicates (0)
3) **Randomisation:** randomised treatment assignment (if a study follows an experimental planting design): Y (1) /N (0)

Studies with low risk of bias score 4-5 for the critical appraisal above. Studies with medium risk of bias will score 1-3. Studies with a score of 0 have a high risk of bias.

We will also record whether studies contain any confounding variables (such as geographic or temporal factors) that could affect study validity.

Rather than excluding high risk of bias studies from the review *a priori*, risk of bias will be explored as a potential effect modifier in the narrative and quantitative synthesis. For each meta-analysis that is conducted, the same analysis will be repeated only using studies with low risk of bias, to assess the reliability of the result of the main meta-analysis.

Data Extraction Strategy

All data will be extracted into a standardised spreadsheet format that will be available as supplementary material for the systematic review. To check for consistency in the extraction of quantitative data, a second reviewer will also perform quantitative data extraction on a randomised subset (10%, or 10 studies, whichever number is largest) of studies.

A piloted data sheet in the format that will be used for all data extraction is available as supplementary material to this protocol.

**Extraction of study metadata**

The following attributes of each study and its methodology will be extracted when available in text, tables or graphs.

- Study type (primary research paper, narrative review paper, meta-analysis, book chapter)
- Date of study
- Study location:
  - Geographical region (continent, country);
  - Climatic region (tropical, dry, temperate, cold/boreal or polar according to Köppen's classification (Peel, Finlayson, & McMahon, 2007));
  - Latitude and longitude of site(s) under study
- Size of plots / stands ($m^2$)
- Number of trees sampled in treatment and control
- Metric of pathogen impact recorded (e.g. % mortality, % foliar damage, no. of stem cankers)
- Age of trees at time of sampling
- Focal tree species on which damage recorded
- Other species of tree present in treatment sampled
- Pathogen species / groups recorded
- Method by which pathogen species are identified (visual assessment in field, microscopic, or molecular methods)

**Extraction of study findings**

For each study, the following summary statistics will be extracted where available either from the main text, tables or graphs:

- Mean pathogen impact in treatment and control
- Measure of variance (e.g., Variance, Standard Deviation or Standard Error) for both treatment and control
- Sample size for treatment and control
- For studies where analysis is by linear regression, Pearson's correlation coefficient and its variance will be calculated from summary statistics using standard equations (Koricheva et al., 2013)

Where data is only available in graphs, the online tool Web Plot Digitizer (https://automeris.io/WebPlotDigitizer/ ) will be used to extract data from images.

For some studies, there may be multiple treatments (for instance, along a tree species richness gradient) that may be compared to the same control plot. The non-independence of such studies will be coded using unique numbers for studies using the same control within each paper, as well as for all studies from the same paper. These identifiers can then be used as random effects in a mixed effect meta-analysis model at the evidence synthesis stage.

**Potential effect size modifiers**

The following additional data will also recorded where possible, to capture information on sources of heterogeneity between studies. This list of effect modifiers was formulated following consultation among the authors, with additional input from expert stakeholders.

1. Focal tree species abundance in mixed stand (proportion or percentage of the focal species in the treatment stand). Inclusion of this moderator will enable us to test whether the effect of mixed stands varies with the level of host dilution within a stand, in addition to the tree species richness.
2. Tree species richness of mixed stand
3. Forest type (broadleaved, conifer or mixed)
4. Susceptibility of associated tree species in mixed stand, where known (e.g. no other susceptible hosts present, vs less susceptible host present, vs more susceptible host present).
5. Whether a study is observational (semi-natural or unplanted forest) or experimental (planted forest).
6. Pathogen group (fungal, bacterial, viral, oomycete)
7. Pathogen specialisation (specialist vs generalist)
8. Pathogen type (endemic vs putative invasive)
9. Rotation type (short rotation vs long rotation forest crop)
10. Tree organ where damage recorded (leaves, vascular system, roots)
11. Study risk of bias (as assessed at the critical appraisal stage: low, medium or high)

**Approaches to missing data**

If any of the quantitative data above is missing from a published study, provided the study has been published within the last two decades, the authors will be contacted to request further data. Authors will be contacted using the contact details on the original paper, or where possible, using contact details from the most recent article on which they are corresponding author. Authors will be given a time frame of six weeks from the period of first contact to provide missing data, should they choose to. In some cases, it may be possible to calculate the necessary statistics required for meta-analysis (effect size and its variance) from the data reported in the published paper by interconversion between effect size metrics using standard equations, or in the case of variance estimates, via extrapolation from the results of other studies (Koricheva et al., 2013). Thus, even if no further data is provided from authors, the study may still be included in the meta-analysis.

**Extraction of summary statistics from raw data sets**

Data from a number of unpublished studies has also been provided from TreeDivNet stakeholders. Additional raw data or summary information may be provided by other stakeholders from the list of contact organisations. Following critical appraisal of these studies, summary statistics for each study will be calculated directly from raw data (mean, variance and sample size for mixtures and monocultures).

<u>Data Synthesis and Presentation</u>

**Narrative Synthesis**

The data will be assimilated into a narrative review highlighting the key results of research in this area, as well as remaining knowledge gaps. Wherever possible, quantitative results from studies will be used to provide evidence for the conclusions of the review. We will tabulate studies along with their metadata in order to explore the distribution of evidence between geographic, climatic regions and critical appraisal groups. Details of this process will be decided once the studies have been collected and the properties of the evidence base have been established.

**Quantitative Synthesis**

In addition to a narrative synthesis, if sufficient quantitative data is available we aim to synthesise results from across studies using meta-analysis. In addition to addressing the primary question by calculating an

overall effect size across studies, we aim to use meta-regression to address the secondary questions, by exploring the sources of heterogeneity in effect size (Koricheva et al., 2013). Depending on the amount of data on each moderator that is available, moderators to be explored using meta-regression may include: degree of host dilution, pathogen specialisation, neighbouring host susceptibility and forest type (see potential effect modifiers above, and hypotheses 2 – 6 below). All analyses will be done in R using the *metafor* package. The effect size metric chosen will depend on the final data set, but is likely to be Hedge's *d* statistic (the bias corrected standardised mean difference) as:
(a) categorical explanatory variables are likely to be the most common in the literature (i.e. comparing monocultures to mixed stands)
(b) it is possible to convert to *d* to/from other statistics that may be available from studies using standard equations (Koricheva et al., 2013)
(c) it is appropriate for use with smaller sample sizes.

**Hypotheses for Meta-analysis**

1) Mixed species stands are overall more resistant to pathogens (have lower pathogen damage or incidence rate).
2) A reduction in host density (increased host dilution) is associated with reduced pathogen incidence in mixed stands.
3) The effect of mixed stands on pathogen incidence is stronger for specialist pathogens than for generalist pathogens.
4) Associational resistance in mixed species stands is stronger (more negative effect size) in stands where associated tree species are of a different type (broadleaved or conifer).
5) Associational resistance in mixed stands is stronger in stands where associated tree species are not susceptible or are less susceptible than the focal species.
6) In stands where associated tree species are more susceptible, the effect of mixtures on pathogen incidence is reversed (associational susceptibility) or not significantly different from the monoculture.

**File drawer problem / Publication bias**

To identify possible publication bias, funnel plots will be produced in *metafor* and investigated for asymmetry (Nakagawa & Santos, 2012). To address the impact of publication bias, a failsafe number will be calculated using Rosenthal's method (Rosenthal, 1979). This represents the minimum number of statistically non-significant, unpublished or missing studies that would need to be added to the analysis in order to make the overall effect non-significant. This will then be compared to Rosenthal's conservative critical value of $5n + 10$. Given that the scope of the review is limited to studies published in English, there may be several relevant studies that will be missing. Addressing the extent of any publication bias will therefore be critical to establishing the validity of the conclusions of the review.

## References


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Appendix I:
**Test Library used in Scoping Searches**

1. Dillen, Mathias, Kris Verheyen, and Christian Smit. 2016. Identity rather than Richness Drives Local Neighbourhood Species Composition Effects on Oak Sapling Growth in a Young Forest. Forest Ecology and Management 380 (November): 274–84. doi:10.1016/j.foreco.2016.09.004.

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Appendix II

**Metadata Form sent to stakeholders from TreeDivNet contributing unpublished data sets**

| **TreeDivNet Pathogen Data for Systematic Synthesis** | |
|---|---|
| **Name:** | **Institution:** |
| **Position:** | **Contact email:** |
| **Contact address:** | **Experiment name:** |
| **Location of experiment (latitude and longitude):** | **Dates of data collection:** |
| **Year planted:** | |
| **Who collected the data?** | **Contact email of the person who collected the data (if different from above):** |
| **Tree species planted in experiment:** | **Tree species sampled for pathogens:** |
| **Please provide a brief description of sampling protocol, including the number of plots sampled and treatment levels, number of trees sampled per plot, number of leaves/shoots sampled per tree.** | |
| **How were pathogens identified (e.g. by eye in the field, microscopically from sampled leaves, molecular methods)?** | |
| **What is the metric of pathogen damage recorded? (e.g. % damage per leaf, number of stem lesions, etc).** | |
| **Further comments** | |

**Appendix III:** List of search strings to be used in each major database queried during the systematic review, with number of hits returned and date searched.

| Database | Search string used | Number of hits | Date |
|---|---|---|---|
| Scopus | ( TITLE-ABS-KEY ( ( tree* OR forest* OR plantation OR wood* OR orchard OR coppice OR shrub OR abies OR acer OR betula OR eucalyptus OR fagus OR fraxinus OR picea OR pinus OR populus OR quercus ) AND ( "mixed species" OR "mixed-species" OR "mixed stand*" OR "tree divers*" OR "forest divers*" OR "tree species divers*" OR "host species divers*" OR "host divers*" OR "community divers*" OR "forest species divers*" OR "divers* stand" OR "divers* plant*" OR "divers* forest" OR "divers* wood*" OR "tree species rich*" OR "host species rich*" OR "forest species rich*" OR polyculture* OR "landscape heterogen*" OR "heterogen* landscape" OR "species mix*" OR "diversity-disease" OR "species composition" ) AND ( pathogen OR disease OR infection OR "root rot" OR "butt rot" OR fung* OR lesion OR canker OR bacteria* OR viral OR virus OR phytophthora OR armillaria OR heterobasidion ) ) ) AND ( LIMIT-TO ( SUBJAREA , "AGRI " ) OR LIMIT-TO ( SUBJAREA , " ENVI " ) ) | 1,744 (limited to Topics: Agricultural/Environmental Science and Biological Sciences) | 13th December 2017 |
| Web of Science (All databases: WoS Core Collection 1945-present, BioSIS Citation Index, Current Contents Connect, Data Citation Index, Derwent Innovations Index, Inspec, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index, SciELO Citation Index, Zoological Record) | TS = (tree* or forest* or plantation or wood* or orchard or coppice or shrub or abies or acer or betula or eucalyptus or fagus or fraxinus or picea or pinus or populus or quercus) AND TS = ("mixed species" or "mixed-species" or "mixed stand*" or "tree divers*" or "tree species divers*" or "forest divers*" or "host species divers*" or "host divers*" or "community divers*" or "forest species divers*" or "divers* stand" or "divers* plant*" or "divers* forest" or "divers* wood*" or "tree species rich*" or "host species rich*" or "forest species rich*" or polyculture* or "landscape heterogen*" or "heterogen* landscape" or "species mix*" or "diversity-disease" or "species composition") AND TS = (pathogen or disease or infection or "root rot" or "butt rot" or fung* or lesion or canker or bacteria* or viral or virus or Phytophthora or Armillaria or Heterobasidion) | 5,703 (limited to Research Areas within Science and Technology: (Agriculture OR Forestry OR Environmental Sciences OR Ecology OR Plant sciences) | 13th December 2017 |
| CAB Abstracts (Database: 1910 – 2017) | (tree* or forest* or plantation or wood* or orchard or coppice or shrub or abies or acer or betula or eucalyptus or fagus or fraxinus or picea or pinus or populus or quercus) AND ("mixed species" or "mixed-species" or "mixed stand*" or (mixed adj1 stand) or mixture* or "tree divers*" or "tree species divers*" or "host species divers*" or "host divers*" or "community divers*" or "forest divers*" or "forest species divers*" or "tree species divers*" or (forest adj1 divers*) or (tree adj1 divers*) or "divers* stand" or "divers* plant*" or "divers* forest" or "divers* wood*" or "tree species rich*" or "host species rich*" or "forest species rich*" or polyculture* or "landscape heterogen*" or "heterogen* landscape" or "species mix*" or "diversity-disease" or "species composition") AND (pathogen or disease or infection or "root rot" or "butt rot" or fung* or lesion or canker or bacteria* or viral or virus or Phytophthora or Armillaria or Heterobasidion) | 2,756 Searching abstract, title, original title, broad terms, heading words, identifiers, cabicodes | 13th December 2017 |