STUDY PROTOCOL

The effects of crop diversity and crop species on biological diversity in agricultural landscapes: a systematic review protocol [version 1; peer review: 1 approved with reservations]

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Abstract
Agricultural intensification is a well-known driver of biodiversity loss. Diversity of crop production over space and time reduces land use intensity and may mitigate impacts on biodiversity while contributing to growing demand for human food and nutrition resources. Crop species are also known to have independent impacts on biodiversity. To date, reviews synthesising our knowledge of crop species and crop diversity-biodiversity links are missing. We will therefore conduct a systematic review by searching multiple agriculture, ecology and environmental science databases (e.g. Web of Science, Geobase, Agris, AGRICOLA, GreenFILE) to identify studies reporting the impacts of crop diversity and crop species on the biological diversity of fauna, flora and microbes in agricultural landscapes. Outcomes will include metrics of species richness, abundance, assemblage, community composition and species rarity. Screening, data coding and data extraction will be carried out by one reviewer and a proportion will be independently conducted by a second reviewer. Study quality and risk of bias will be assessed. Evidence will first be mapped by species/taxa then assessed for further narrative or statistical synthesis based on comparability of results and likely robustness. Gaps in the evidence base will also be identified with a view toward future research and policy directions for nutrition, food systems and ecology.

Keywords
crop diversity, intercropping, crop rotation, agricultural management, biodiversity, species richness, abundance
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Competing interests: No competing interests were disclosed.

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1. Background
Land use and land use change are recognised as the primary drivers of biodiversity loss. These factors, together with crop species and related management and production cycles, determine the intensity of agricultural management. Agricultural intensification factors that have been well researched in relation to biodiversity include landscape heterogeneity, use of pesticides and fertilisers, and ploughing. Crop diversification has been proposed as a management practice that may reduce some of the environmental impacts of modern farming related to fertiliser and pesticide use and therefore mitigate food production-biodiversity trade-offs – namely, that conventional high-input intensification of agricultural land use reduces conversion of natural habitats but also decreases biodiversity.

Crop diversity has spatial and temporal dimensions. Practices such as mixed cropping or intercropping characterise agricultural diversity in space. Rotation of crops, or the practice of growing different crops in the same field, rotated seasonally or annually, provides agricultural diversity over time. Increased crop diversity over both space and time is associated with improved soil health and pest control, decreased erosion, and increased nutrient cycling. However, relationships between crop diversity and the biodiversity of flora, fauna and soil microbes are less clear and synthesis of the current literature may provide useful insights to help inform the debate on land use trade-offs related to future food production.

Differences in crop species are also known to have independent impacts on biodiversity, for example, that of wheat on soil microbial diversity or fruit orchards on bird abundance. Evidence of these relationships has not yet been mapped or synthesised. Understanding the relationships between crop species and biodiversity – even if mediated by agricultural intensity – may help support the sustainable increase of agricultural production in coming decades. For purposes of this study, crop species are defined as crops cultivated for human and animal use or consumption including food, feed, cover crops, fibres, fuels, and grasslands/herbage for pasture. Whilst within-species genetic diversity of crops, including wild relatives, is very important to future breeding efforts due to potential benefits such as nutritional content or resilience to environmental stress, it is beyond the scope of this review and will not be considered.

Biodiversity is complex and no single metric can assess its multiple dimensions including genetic, species, functional and ecosystem diversity, as it exists over time and space. Commonly used metrics include species extinction and extinction risk, species richness (the number of species in a grid), abundance (the number of individuals per species), and community composition or assemblage of species in a given grid. Rare species richness and relative species rarity are also thought to capture aspects of biodiversity related to functional and phylogenetic diversity. These measurements are practical and individually capture important, if incomplete, dimensions of biodiversity; consequently, they are also the most used in the environmental sciences. This is the first systematic literature review to examine and synthesise literature on the relationship between crop diversity and crop species on common metrics of biodiversity.

2. Aim and objectives
The aim of this review is to answer the primary research question: “What are the effects of spatial and temporal crop diversity and of individual crop species on the biological diversity of fauna, flora and microbes in agricultural landscapes?”

Secondary questions to be answered by this study include:

- Are there trends in the response of biodiversity to crop diversity across different taxonomic groups or biomes?
- Which species or taxonomic groups are most affected by crop diversity?
- Which crop diversification practices have the strongest effects on biodiversity?
- What evidence exists of the effects of crop species on biodiversity?
- What are the hypothesised causal pathways by which crop diversity or crop species may have effects on biodiversity?

The study objectives are:

- To identify, assess and summarise studies that have estimated the impacts of crop diversity and crop species on biodiversity among flora, fauna and microbes (bacteria, fungi, algae and protozoa).
- To synthesise evidence of the impacts of spatial and temporal crop diversity on biodiversity.
- To identify trends in the response of biodiversity to crop diversity across different taxonomic groups or biomes.
- To map evidence of the impacts of crop species on biodiversity.
- To highlight research gaps.

3. Methods
3.1. Search strategy
Due to the transdisciplinary nature of the research, multiple databases covering the fields of environment and ecological sciences and agriculture will be searched, namely: 1) Web of Science Biological Abstracts, Reports, Reviews, and Meetings (BIOSIS) Citation Index (Clarivate Analytics), 2) Web of Science, Science Citation Index (Clarivate Analytics), 3) Commonwealth Agricultural Bureaux (CAB) Abstracts (Ovid), 4) Geobase (Ovid), 5) International System for Agricultural Science and Technology (AGRIS) (UN Food & Agriculture Organisation), 6) GreenFILE (Ebsco), 7) AGRICOLA (AGRICultural OnLine Access) (USDA National Agricultural Library), 8) Northern Light (Ovid), 9) Open Grey (INIST-CNRS), and 10) Dissertations & Theses Global (ProQuest). Review exposures and outcomes are listed in Table 1.
This review is global and no geographical limitations will be used. Abstracts in English will be reviewed and, following screening, full text articles in languages other than English will be translated. Grey literature databases will also be included to minimise publication bias and increase the comprehensiveness of the review.

Inclusion criteria:
- Full-text articles
- Controlled experiments, observational studies, modelling studies
- Quantitative studies that quantify the impacts of crop diversity or crop species on one of the following biodiversity metrics: extinction, extinction risk, species richness, population abundance, assemblage, community composition, rare species richness/abundance or relative rarity
- Exposures measure crops grown or cultivated for human and animal use or consumption including food, feed, cover crops, fibres, fuels, and grasslands for pasture/grazing
- Outcomes measured among fauna, flora, and microbes, namely: bacteria, fungi, algae and protozoa
- All years

The following controls or comparators will be included:
- Spatial crop diversity (mixed, pattern cropping) compared to monoculture
- Temporal crop diversity (rotational) compared to lack of rotation
- Crop species compared to
  - other crop species; or
  - mixed natural/agricultural vegetation (e.g. agroforestry)

Exclusion criteria:
- Review articles with no original results presented
- Qualitative studies
- Exposure effects presented solely in combination with landscape composition or other agricultural management effects e.g. non-crop vegetation or structures (except grasslands used for pasture/grazing), no-till, etc
- Comparators for crop species exposures: natural, unaltered landscapes and rangeland

A set of complete search terms for the Web of Science database is available as extended data. Key concepts are captured by three topics: 1) crop diversity, 2) crop species and 3) biodiversity metrics. Use of “Near/15” will link exposure-related terms to agricultural landscapes, while “Near/5” specifies precise exposure and outcome terms observed in the literature and close variants thereof. In addition to terms identified in preliminary searches, the Food and Agriculture Organization (FAO) Indicative Crop Classification (ICC) was used to help construct the crop species search terms, and the BIOSIS Citation Index list of taxa notes were used to help construct the list of biodiversity search terms. The search strategy has been reviewed by an experienced librarian with no other collaboration on the project.

3.2. Screening, data coding, and data extraction
To screen and extract data, search results will be downloaded to an Endnote database. Duplicates will be removed, first electronically (exact match only), then manually to account for misspellings and slight differences. Titles will first be screened for inclusion and exclusion criteria, then abstracts, and finally full text papers (CM). A second independent reviewer (FH) will screen 10% of titles, abstracts and full texts. Discrepancies will be discussed and agreed by consensus, with a third reviewer if necessary (RG). If there are major differences between included texts, the second reviewer will screen a further 10% of articles and discrepancies will be reconciled as above. Data will be coded and extracted by the primary reviewer (CM); a second reviewer (FH) will independently code and extract data for 10% of full texts included. For the papers identified for inclusion in the review, data coded and extracted will include the following: authors, year, publication, study location, study design, scale, biodiversity metric, species/taxa (super taxa, taxa, organism classifier, organism name), crop species, crop diversity, duration of intervention, number of crop rotations, effect sizes, standard deviations, sample sizes, biome, ecoregion, climatic zone, field size, and other agricultural management, landscape, environmental and climatological factors. If data is not available directly in the text, the corresponding author will be contacted and data requested.

3.3. Data management
All search results including titles and abstracts will be exported to and managed within Endnote. Complete results for each database will be maintained, as will duplicates excluded
and the results of each stage of screening. Full texts reviewed and excluded will be categorised by reason for exclusion with notes maintained using the designated field in the Endnote record. If a full text cannot be accessed, the corresponding author will be contacted and up to two contact efforts will be made. A contact record sheet will be kept with author names and study title, email addresses, dates(s) of contact, and results of contact.

A pilot data coding and extraction form will be developed at the outset of the data extraction process. Data from the first five full text papers included in the review will be extracted using the form. It will then be adapted as needed to best reflect common data formats and data re-extracted as required from the first five papers. This process will be repeated until no further adaptation is required. Each form with data extracted will be tracked and dated. The final data extraction form will then be given to the second independent reviewer (FH) and data extraction will be conducted for 10% of the full texts included in the review.

If a corresponding author is contacted to obtain data, up to two contact efforts will be made and tracked using the contact record sheet process previously outlined. If no new contact information can be identified and there is no response from the author, or if the author declines to share data, the study will be excluded from further analysis. This will be noted in the study limitations in the final review report.

### 3.4. Study quality and risk of bias assessment

Adapting the quality assessment tool developed by the Critical Appraisal Skills Programme (CASP)\(^\text{26}\), the following questions will be used to assess each study meeting the full inclusion criteria:

- Was there a clear description of the crops evaluated?
- Was there a clear description of the biodiversity metrics evaluated?
- Was there a clear description of the species and taxa evaluated?
- Was a clear description given of field conditions and agricultural practices used?
- Was a clear justification given for conducting a study in a particular area – including a description of agricultural conditions?
- Were crops under the “intervention” compared to an appropriate and comparable baseline group or situation?
- Were the methods of measuring the agricultural exposure(s) clearly described?
- Were the methods of measuring the biodiversity outcome(s) clearly described?
- Are sufficient data presented to support the findings?
- Were analyses described in detail?
- Did the researchers critically examine their potential biases during measurement, analysis and selection of data for presentation?

Papers will be scored between 1–11, with 1 mark given for each ‘Yes’ above. To assess risk of bias, the Environmental-Risk of Bias tool will be adapted and a low, high or unclear mark will be given for each of the following categories: selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias related to study design\(^\text{26}\). Quality and risk of bias assessment results will be reported for all papers, and any papers scoring less than 8 and/or presenting insufficient data to support the findings will be excluded from further synthesis. The quality assessment review will be done by the first reviewer (CM) and a second reviewer (FH) will independently assess 10% of the full texts included.

### 3.5. Data synthesis

Data synthesis will aim to explore both patterns and dispersion in the data. It will first be conducted using the following three steps: 1) complete a textual description of studies, 2) tabulation of studies by groups and clusters, and 3) preliminary synthesis and development of a common results rubric. To tabulate studies, results will be grouped by 1) biodiversity metric, followed by 2) exposure, 3) species/taxa, and 4) control/comparator. Species/taxa may be combined where appropriate up to the super taxa level e.g. ants and spiders re-categorised as arthropods. Measures of exposure such as all-crop diversity (e.g. over both space and time) or crop species by vegetation structure (e.g. orchard crops) may also be grouped subject to similarity of the comparison groups.

**Evidence mapping and narrative synthesis**

Results for certain data groups (exposures: crop species; outcomes: extinction, extinction risk, assemblage, community composition, relative rarity) may be insufficient in number and/or highly heterogeneous. Therefore quantitative synthesis will be infeasible or unlikely to be robust. In such event, results will be described by heat map, identifying the number of studies providing evidence by outcome, exposure and taxa or super taxa (population). If results are of a sufficient number but highly heterogeneous, thematic analysis will be conducted using narrative approaches and finally, conceptual mapping will be conducted to explore relationships between the findings.

**Quantitative analysis**

Two outcomes will be considered for quantitative analysis: species richness and abundance since these metrics tend to be those most often measured. By taxa category, statistical summary will be explored if there are a sufficient number of study results which also report the effects of the same exposure. Further criteria for statistical summary will include use of experimental and observational study designs and availability of variance estimates and sample sizes. All data from the extraction form will
be imported for handling into the R environment. RStudio 3.5.0 is a free software environment for statistical computing and graphics\(^27\). Using the R package metafor (version 2.1.0), effect sizes for species richness and abundance will be calculated as response ratios (the magnitude of difference between groups), which do not require measures of within-group variance and are commonly used in the ecological sciences because results from different study designs, scale and taxonomic groups may be appropriately combined\(^28\). Random effects meta-analysis models will also be used to account for heterogeneity and study identifier will be set as the random effect. If present in a sufficient number of studies, agricultural management covariates will also be included in the models. The estimated range of true effects i.e. differences in effects observed, will be reported using forest plots and confidence intervals. Sensitivity analyses will also be conducted by comparing results of full models with those: 1) without observational studies and 2) of low study quality (defined as a score of <9 marks after following the procedure outlined in section 3.4).

Data synthesis will be conducted by the first author (CM) and reviewed by other contributors.

4. Sources of bias

Reviewer bias: Inclusion and exclusion criteria may be interpreted differently. A third reviewer will be identified if discrepancies arise between the first two reviewers.

Publication bias: If statistical summary is conducted, Rosenthal’s fail safe number – the number of unpublished studies reporting no evidence of effects that would need to be added to a summary analysis in order to change the results – will be calculated to indicate the credibility of the results. If this is infeasible due to study heterogeneity, then lack of ability to estimate publication bias will be acknowledged as a limitation of the study in the final reporting.

Selective reporting bias: Because it is not common practice in the environmental sciences to register experimental study protocols prospectively, it is not possible to evaluate within-study selective reporting. This limitation will be acknowledged in the final systematic review report.

Inconsistent outcome definitions and methods: There are differences in the way that biodiversity metrics (e.g. relatively rarity) are measured, defined or calculated by ecological researchers. Differences will be carefully considered prior to data synthesis.

5. Outputs

Results of the analysis will map and/or synthesise evidence of the effects of crop diversity and crop species on a variety of different taxa and biodiversity metrics. Gaps in the literature will also be identified, with a view toward future research and policy directions for nutrition, food systems and environment. Key outputs from the systematic review will include a full literature database on the effects of crop diversification and crop species on biodiversity, tables of study characteristics and of synthesised analyses and/or evidence map and narrative summarising results.

6. Ethics and dissemination

This review will not use data collected from human subjects. An application for ethical approval has been submitted to the London School of Hygiene & Tropical Medicine Ethics Committee (ref 17546). Findings will be published in a peer-reviewed journal.

7. Study status

The study protocol and search strategy have been completed; as of publication, searching has not yet begun.

8. Data availability

Underlying data

No data is associated with this article.

Extended data

Figshare: Extended Data File 1 Search Terms.docx. https://doi.org/10.6084/m9.figshare.8290004.v1\(^22\)

This project contains the following extended data:

- Extended Data File 1 Search Terms.docx (Web of Science BIOSIS Citation Index systematic review search terms)

Reporting guidelines


Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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References


24. Web of Science Group: BIOSIS Citation Index. Clarivate Analytics; 2019. Reference Source


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General comments

Biodiversity loss across all taxa and scales is a hugely important topic, especially in the light of global change, ecosystem resilience and resistance. At the same time, agricultural intensification plays a major role in species declines, necessitating a move towards biodiversity-friendly farming practices. The value of crop diversity and crop type for soil quality and productivity has long been shown, and numerous studies and literature reviews highlight the benefits for biodiversity as well. However, most of these papers concentrate on specific taxonomic groups or study systems, and quantitative syntheses of crop diversity effects are mainly lacking. For instance, two published quantitative reviews by Dassou & Tixier (2016) and Letourneau et al. (2011) focus primarily on abundance and/or richness measures of herbivores and predators, ignoring other taxonomic groups. Therefore, quantitatively summarising the effects of crop diversity and crop type on different taxa and other aspects of biodiversity such as extinction risk is an essential step forward.

This study protocol proposes a method to screen and synthesise literature related to the benefits of crop diversity and crop species on biodiversity in agricultural landscapes. The authors propose to review both peer-reviewed and grey literature databases using a variety of different search terms, then applying thorough quality and bias assessment of potential studies, before narratively and quantitatively synthesising the effects.

While acknowledging the great importance of the proposed review, there are a few general shortcomings of the study protocol that I would like to address.

Next to temporal crop diversity (crop rotations), the authors mention mixed cropping or intercropping as a type of spatial crop diversity included in the review. What about crop diversity on a landscape scale, i.e. not on the same field? This spatial crop diversity can have positive effects on biodiversity and ecosystem services (e.g. Fahrig et al. 2015; Hiron et al., 2015; Palmu, Ekroos, Hanson, Smith, & Hedlund, 2014).
Redlich, Martin, & SteffanDewenter, 2018). It also plays an important role in agricultural policy, for example in Europe, where farmers are obliged to increase the number of crops grown on a farm.

On the other hand, I slightly struggle with the term ‘crop species’ used throughout the paper. For two reasons:
First, I think that the term ‘crop species’ is slightly misleading in this context, as it could also imply the richness of crop species, which is obviously not the intention. Using a term such as ‘crop type’ or ‘crop identity’ may be helpful.
Second, quantitatively assessing the effect of ‘crop species’ is non-trivial, because there are numerous crops grown worldwide (and there is no geographical restriction applied in this review), and each crop could have been compared to numerous other crops or mixed natural-agricultural systems. These different crop-crop combinations, however, can only be assessed with difficulty and a lot of effort, and most likely not using quantitative measures. Rightly, the authors expect this issue and propose narrative and mapping approaches instead. They also suggest the grouping of crops whenever needed or possible (e.g. by crop characteristics/functions). To me, the latter approach is most valid and useful, as different studies have shown the benefits of using functional groupings over crop species per se. In this case, however, the title and use of the term ‘crop species’ is misleading.

As a last general comment, the terms “exposure” and “outcome” are not normally used in ecology (which is the primary field of research this review focuses on) to describe drivers (crop diversity and crop type) and response variables (biodiversity metrics). In ecological studies, the ‘outcome’ would be that effects are either positive, neutral or negative for biodiversity.

Apart from these and some minor points (see below) that may require some clarification, I recognize the value of the planned review and the generally strong and thorough design of the study protocol. I am very much looking forward to seeing some first results of a quantitative synthesis of crop diversity effects on biodiversity.

Section-specific comments

Abstract
I missed a sentence about why the loss of biodiversity is considered a problem, especially in agricultural systems.

“Crop species are also known to have independent impacts on biodiversity.” This sentence is not very clear, especially the meaning of ‘crop species’. I guess the authors aim to say that depending on the crop type, effects on biodiversity can differ (independently of overall crop diversity benefits).

“… and a proportion will be independently conducted by a second reviewer.” The idea that the second reviewer takes over the task of quality control only becomes clear when reading the methods section.

Background
I missed the link between land use (which can include a lot of changes not only related to agriculture) and agricultural intensification.

“These factors, together with crop species and related management and production cycles, determine the intensity of agricultural management”. As in the abstract, the exact meaning of ‘crop species’ in this context is not clear to me. I assume the authors mean to say that the type of crop grown also determines the management practices and crop rotations required, and therefore makes farming more or less
intensive (e.g. oilseed rape farming requires high insecticide inputs, while winter wheat needs large amounts of fertilizer inputs and is often grown in short rotations).

I agree that land use and land use change affect biodiversity, but not necessarily why “these factors” should determine the intensity of agricultural management, unless a change in land use involves growing more management intensive crops, monocultures etc.

What is the difference between ‘rotation of crops’ and ‘the practice of growing different crops in the same field, rotated seasonally or annually’?

Aim and objectives
Secondary questions and study objectives are redundant (e.g. “Are there trends in the response of biodiversity to crop diversity across different taxonomic groups or biomes?” and “To identify trends in the response of biodiversity to crop diversity across different taxonomic groups or biomes”). I recommend focussing on study objectives, as these more clear.

Methods
Methods, statistical analyses and visualisation options seem to be appropriate for the purpose of this review.

I very much like the fact that the literature search is not restricted to scientific literature, but also grey literature and dissertations/thesis. While some may argue that the quality of such literature may not compare to peer-review research articulated, I am a strong advocate for recognizing the value of research that does not end up being published in scientific journals, as an abundant amount of relevant evidence otherwise gets lost. I also believe that applying quality and bias assessment as described later will ensure comparable standards for both peer-reviewed and grey literature.

The list of databases and the search terms used to find papers assessing drivers and biodiversity metrics seem to be quite comprehensive. The authors make huge efforts to double-check the screening and coding process, ensure the study quality and reduce the risk of methodological/design/publication bias. Notwithstanding my comments above about pooling crop species, using narrative description and mapping would be a nice (but time-consuming) way to deal with insufficient/heterogeneous data. I also very much appreciate the protocol for recoding every step of the review process and data acquisition, as well as applying the Critical Appraisal Skills Programme used in healthcare science to an ecological study!

Some other comments:
- For biodiversity metrics: I suggest to stick with the same term and order in the table and text (e.g. always “relative species rarity”).
- What does the inclusion criterion ‘All years’ refer to?
- “comparators” does not sound like the right word in this context. “Control” or even “reference/baseline crop/habitat” may be more suitable.
- Again, what about across-field spatial crop diversity? In this case the control would be a landscape with low spatial crop diversity (e.g. multi-crop landscapes compared to landscapes with only a few crops grown).
“Exposure effects presented solely in combination with landscape composition or other agricultural management effects e.g. non-crop vegetation or structures (except grasslands used for pasture/grazing), no-till, etc" This is not clear to me.

Options to shorten and combine: “A contact record sheet will be kept with author names and study title, email addresses, dates(s) of contact, and results of contact. If no new contact information can be identified and there is no response from the author, or if the author declines to share data, the study will be excluded from further analysis”.

References

Is the rationale for, and objectives of, the study clearly described? Yes

Is the study design appropriate for the research question? Yes

Are sufficient details of the methods provided to allow replication by others? Yes

Are the datasets clearly presented in a useable and accessible format? Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Agroecology, ecosystem services and biodiversity.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.