Kostov *et al. Environmental Evidence* 2014, **3**:11
http://www.environmentalevidencejournal.org/content/3/1/11

ENVIRONMENTAL EVIDENCE

SYSTEMATIC REVIEW PROTOCOL Open Access

Are soil microbial endpoints changed by Bt crops compared with conventional crops? A systematic review protocol

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Abstract

Background: *Bt* crops have raised environmental concerns over consequences for sustainability of soil biodiversity and ecosystems services in agricultural land. As *Bt* crops contain insecticidal proteins potential interactions with non-target organisms are of major concern for the risk assessment. In recent years, numerous field and laboratory studies have been conducted to assess the potential adverse effects of the *Bt* crops on different non- target organisms including microorganisms living in the soil. Soil microorganisms include primarily bacteria and fungi. They interact with plants and animals and play significant roles in nutrient cycling and energy flow in soils and are actively involved in chemical and biological processes. It is likely that any changes accruing in soil will affect soil microorganisms, and so their abundance/diversity and activities can be used as indicators for many aspects of soil quality, and thus for the assessment of the potential effects *Bt* crops on the environment.

Methods: The key review question is: Are abundances/diversity/activities of soil microorganisms changed by *Bt* crops compared with conventional crops? For this purpose relevant literature will be collected systematically through a comprehensive search strategy in a number of general, specialized and personal databases. The search terms will define the locality or type of laboratory/glasshouse study – the field, soil, rhizosphere, the populations - types of soil microorganisms, the exposure - types of *Bt* proteins, the assessed outcome -abundances/diversity/activity of microorganisms and the crop, will be used for retrieving of relevant studies. We present the criteria against which studies will be included in the review and how they will be assessed. These criteria include appropriate study designs and comparators. This protocol outlines the type of analyses that will be performed to assess bias of the selected studies and if co-variables describing the heterogeneity of the studies introduces biases. The synthesis will be performed as a quantitative synthesis combining the magnitude of potential effects from the individual studies. The synthesis will include assessments of heterogeneity related to the studied abundances/ diversities/activities, types of exposure, methodology, comparators and publication bias.

Keywords: Soil microorganisms, *Bt* crops, Risk assessment, Cry toxins, Bacillus thuringiensis, δ-endotoxin, Soil processes

Background

The debate over genetically modified (GM) plants is continuous in the European Union as a consequence of high public sensitivity and complexity of safety issues which they raise. Among the primary concerns are the effects on the environment associated with potential gene flow, weediness or invasiveness of GM plants and possible interactions with non-target organisms. Government regulators in many parts of the world require an assessment of environmental impacts prior to commercialization of GM crops. For that reason many studies analysing the interactions between GM plants and the environment have been carried out, which are of particular interest to professionals - risk assessors or regulators, as well as for many public and non-governmental organizations [1].

The following protocol describes the methodology for conducting a systematic review and evidence synthesis, which will be performed within the EU funded project GRACE (GMO Risk Assessment and Communication of Evidence). The project aims to explore the existing data

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about potential health, environmental, and socio-economic impacts of GM plants in a comprehensive, systematic and transparent manner. The primary goal of the project is to address issues of high public interest, therefore different stockholder groups were invited to take part in the formulation and prioritization of the questions to be reviewed and the review methodology as well. Through face-to-face consultation and online questionnaires several high priority questions about the environmental impact of GM plants were selected, among which is the one related to the interaction of GM crops producing toxins from the bacterium *Bacillus thuringiensis* (*Bt*) with non-target soil microorganisms.

The technology for genetic modification of plants provides a tool for crop breeding and has been applied for the development of varieties with novel or improved traits. Insect resistance has been achieved via introduction of *Bt* genes and is the second most distributed GM trait worldwide after the herbicide tolerance. The only GM plant which is cultivated commercially in the EU is maize designed to produce *Bt* toxin - Cry1Ab, which provides protection against corn borers (Lepidoptera). GM crops producing different types of *Bt* toxins against other pest insects or in combination with other GM traits are also in the industry pipeline [2,3]. Because *Bt* crops contain insecticidal proteins potential interactions with non-target organisms are of major concern for the risk assessment [4]. In recent years, numerous field and laboratory studies have been conducted to assess the potential adverse effects of the *Bt* crops on different non-target organisms, including microorganisms living in the soil. Soil microorganisms include primarily bacteria and fungi. They interact with plants and animals, play significant roles in nutrient cycling and energy flow in soils and are actively involved in chemical and biological processes. It is likely that any changes accruing in soil will affect soil microorganisms, and so their abundance/diversity and activities can be used as indicators for many aspects of soil quality [5].

There are two possible ways that the *Bt* crop can influence soil living organisms: firstly, directly through root depositions, as exudates, cells and mucilage and through litter that contains Cry toxin and/or through unintended changes in the plant due to the genetic modification; and secondly, indirectly through the changes in agricultural management practices related to the genetic modification, e.g. changed insecticide regimes. The major concern is related to soil microbial ecology and that any effects of *Bt* crop cultivation on soil microorganisms may affect soil ecosystem functioning.

Systematic review and meta-analysis have been applied routinely for synthesis of data from medical studies assessing the risk or benefits of treatments or drugs. However the potential to use this methodology for quantitative synthesis of data from impact assessment of GM plants has also been recognized [6]. Meta-analysis of field and laboratory studies assessing the effects of *Bt* crops to non-target organisms have been performed already [7-11]. Most of them, however scrutinize exclusively non-target arthropods and their communities. Although comprehensive literature reviews exists [12-16] a quantitative synthesis of evidence, with a quality control as required for an SR, of the effects of *Bt* crops on soil microorganisms is still missing. It will produce firm evidence-based conclusions about impacts on biodiversity of soil living microorganisms and to impacts on the soil microbial ecology in general. The approach to the systematic review as detailed here is performed in compliance with guidelines of CEE [17].

Objective of the review

This systematic review aims to synthesise the empirical evidence on the potential effects of *Bt* crops to soil microorganisms and their activities.

The review question (RQ) asked by the present systematic review protocol is:

> Are soil microbial endpoints changed by *Bt* crops compared with conventional crops?
> The question has the following components;

Subject populations

Soil microorganisms, including bacteria and fungi, and their associated activities.

Exposure

Genetically modified *Bt* crops and their concomitant farming practice through the soil environment and in the rhizosphere.

Comparator

The near-isogenic line and their concomitant farming practice through the soil environment and in the rhizosphere.

Outcome

Changes in abundances/diversity and activities of the subject populations contrasted with the comparator.

Methods

The systematic review methodology describes the approach which will be used to find and analyse original articles containing data from field experiments or glasshouse assessing the effects of the *Bt* crop cultivation to soil microorganisms.

Search strategy

The aim of the search is to find studies containing data primarily from field experiments assessing the effect of

Bt crop cultivation to soil microorganisms. However glasshouse studies and studies performed in micro- or mesocosms with field collected soil will also be included. The main approach will be to conduct comprehensive electronic searches in web databases, search engines for scholarly literature and specialized databases. Additional source will be the personal data collected by experts and stakeholders from the project GRACE network. In addition, the reference lists of related review papers and datasets from previously conducted reviews will be checked manually for the presence of relevant studies.

Search terms

Search terms defining the locality or type of laboratory/glasshouse study – the field, soil, rhizosphere , the populations - types of soil microorganisms, the exposure - types of *Bt* proteins, the assessed outcome -abundances/diversity/activity of microorganisms and the crop, will be used for retrieving of relevant studies. The search terms will be organised in strings which will be modified according to the requirements of each bibliographic database. The used search strings and the results will be documented and presented as an Additional file 1 of the systematic review.

The following search terms will be used:

Locality term - soil* OR field* OR rhizo OR meso* OR micro* or glass* or lab*
Population terms - microorg* OR microb* OR bacteri* OR fung* OR minera*
Exposure terms - Cry1* OR Cry2* OR Cry3* OR thuringiensis OR Bt OR endotoxin
Outcome terms - population* OR biomass OR communit* OR diversity OR respirat* OR enzym* OR decomposition OR biogeochem* OR nitrogen OR immobili* OR coloni* OR abundance OR richness
Plant terms - transgen* OR maize Or Corn OR Zea OR cotton OR rice OR soybean OR eggplant OR potato OR poplar

Scoping exercise

A search exercise to determine the scope and relevance of the search strings to be used in the systematic review was performed. Pilot searches were conducted using three platforms: Web of knowledge, Scopus and CAB abstracts. Prior to the pilot search, 19 studies which contained the necessary elements for inclusion in the systematic review were identified by simplified and focused search strings in the Web of knowledge platform. They were used as references to evaluate the relevance of the results from the pilot search. The content of the pilot search strings was modified by including or excluding terms until all 19 articles (if present in the database) were found among the hits and in the same time have not resulted in excessive amount of irrelevant studies. The search strings which produced comprehensive and the most relevant results in the three platforms are shown in Table 1.

Web databases

Web databases containing scientific literature including theses, books, abstracts and articles will be searched using the defined strings. The following search service providers and bibliographic databases will be used:

- Web of knowledge - search service including the following citation databases and platforms:
- Web of Science® - platform which consist of nine databases containing scholarly journals, books, book series, reports, conferences, and more.
- BIOSIS Citation IndexSM - comprehensive reference database for life science research which includes cited references to primary journal literature.
- MEDLINE® - database of the U.S. National Library of Medicine (NLM) contains over 12 million records of journal articles in all areas of the life sciences.
- CAB Direct - platform for access to all CABI database subscriptions.
- Scopus - large abstract and citation database of peer-reviewed literature.
- AGRIS - Information system for the agricultural sciences and technology
- All searches will be performed in English; however no restriction for language or year of publication will be made. In that way all studies which have published an abstract in English will be within the scope of our search. Original full text in either English or German will be included in the analysis. The search results from each of the used sources will be saved and citations will be imported in EndNote citation manager software. All duplicates will be removed and a list containing the accumulated results will be created and uploaded to the open-access database CADIMA (Central Access Database for Impact Assessment of Crop Genetic Improvement Technologies).

Web search engines

The following search engines will be used:
Google Scholar (http://scholar.google.bg/)
Jstor (http://www.jstor.org/)

The same searching strings as described above will be used. The first 200 hits will be checked for relevance. The links will be followed once from the original hit.

Specialized databases

Center for Environmental Risk Assessment. Bibliography database (http://cera-gmc.org/index.php?action=bibliography_database) Bibliosafety. The Biosafety Bibliographic

Table 1 Pilot search results (searches conducted 29.04.2014)

Platform	Search string	Total records	Reference papers
Web of knowledge	Topic = (soil* OR field* OR rhizo* OR meso* OR micro* or glass* or lab*) AND Topic = (microorg* OR microb* OR bacteri* OR fung* OR minera*) AND Topic = (cry1* OR cry2* OR cry3* OR thuringiensis OR bt OR endotoxin) AND Topic = (population* OR biomass OR communit* OR diversity OR respira* OR enzyme* OR decomposition OR biogeochem* OR nitrogen OR immobili* OR coloni* OR abundance OR richness) AND Topic = (transgen* OR maize OR corn OR Zea OR cotton OR rice OR soybean OR eggplant OR potato OR poplar)	457	18 from 19
Scopus	TITLE-ABS-KEY(soil* OR field* OR rhizo* OR meso* OR micro* or glass* or lab*) AND TITLE-ABS-KEY(microorg* OR microb* OR bacteri* OR fung* OR minera*) AND TITLE-ABS-KEY(cry1* OR cry2* OR cry3* OR thuringiensis OR bt OR endotoxin) AND TITLE-ABS-KEY(population* OR biomass OR communit* OR diversity OR respira* OR enzyme* OR decomposition OR biogeochem* OR nitrogen OR immobili* OR coloni* OR abundance OR richness) AND TITLE-ABS-KEY(transgen* OR maize OR corn OR Zea OR cotton OR rice OR soybean OR eggplant OR potato OR poplar)	536	19 from 19
CAB abstracts	Topic = (soil* OR field* OR rhizo* OR meso* OR micro* or glass* or lab*) AND Topic = (microorg* OR microb* OR bacteri* OR fung* OR minera* AND Topic = (cry1* OR cry2* OR cry3* OR thuringiensis OR bt OR endotoxin) AND Topic = (population* OR biomass OR communit* OR diversity OR respira* OR enzyme* OR decomposition OR biogeochem* OR nitrogen OR immobili* OR coloni* OR abundance OR richness) AND Topic = (transgen* OR maize OR corn OR Zea OR cotton OR rice OR soybean OR eggplant OR potato OR poplar)	1607	17 from 19 (2 articles are not present in the database)

Database http://bibliosafety.icgeb.org/) Crop life. Database on Safety and Benefits of Biotechnology (http://biotechbenefits.croplife.org/) Biosafety Clearinghouse. Biosafety Information Resource Centre (http://bch.cbd.int/database/resources/) The same searching strings as described above will be used.

Personal datasets

Experts and stakeholders from the consultation network created within the GRACE project will be asked to provide data relevant to the topic. The complete list of articles received by personal communication and the source will be recorded and will be included in the appendix of the systematic review.

Manual search

Literature datasets or databases from other reviews related to effects of *Bt* crops on soil microorganisms, as well as the reference lists of the articles found within the electronic search will be searched manually.

Article screening

Study inclusion criteria

In order to be included the article needs to fulfil each of the following criteria:

- **Relevant organisms or activities:** Soil microorganisms, including bacteria, fungi and microorganisms at other taxonomic levels and their activities, including respiration, decomposition, biogeochemical cycling, enzymatic activities, mineralisation, immobilisation, nitrogen turnover, root colonization etc.
- **Relevant exposure(s):** Exposure to genetically modified *Bt* crops and their associated farming practice through the soil environment and the rhizosphere.
- **Relevant comparator(s)** (if appropriate): Ideally the near-isogenic line, however less related varieties will be accepted. In field experimental design allowing for any of the following comparisons will be accepted:
  - *Bt* with non-*Bt* plots, neither of which received any additional insecticide treatments.
  - Unsprayed *Bt* plots with non-*Bt* plots that received insecticides.
  - *Bt* with non-*Bt* crops when both are subject to insecticide treatments.
- **Relevant outcomes:** Changes in organism abundance, diversity or activity.
- **Relevant study design:** The studies must ideally be conducted under field conditions designed according to any block or plot randomization method, however other designs might be accepted if the design is explained in details and makes statistical analysis valid. However, glasshouse studies and studies performed in micro- or mesocosms with field sampled soils will also be included, if they apply to the inclusion criteria.
- **Data:** Quantitative data should be available. They include binary and categorical data.

Applying study inclusion criteria

The selection of studies which fulfil the inclusion criteria will be performed at three stages by two reviewers independently. At first, articles will be selected by their title to remove highly irrelevant studies from the overall search results, followed by the second stage in which the inclusion criteria will be applied against the abstracts of the articles. If there is doubt or lack of enough information for judgement of whether the article will pass to the next stage the full-text will be analysed to ensure it meets the inclusion criteria.

At the beginning of the abstract phase, after the first 100 publications are processed, tests for consistency between two reviewers will be made using Kappa statistics. If the Kappa value is less than 0.6 the inconsistencies will be discussed and the criteria for inclusion will be adjusted taking into account the main reasons for disagreement.

In the third stage, the articles will be reviewed in full text for the presence of each of the elements needed for the inclusion. The reasons for including or excluding of each study at that stage will be recorded.

Study quality assessment

As specified above a study must have

- An appropriate comparator (e.g. isogenic or near isogenic line)
- experimental procedures which address the microbial abundance/diversity/activity without bias
- preferentially an experimental design involving replication and randomisation

The lack of any significant outcome between the *Bt*-crop and the comparator does not exclude any study, and is not a selection criterion.

Studies which fulfil the inclusion criteria will be assessed for possible bias of one reviewer and a second reviewer and studies that have been excluded will be reassessed by a second reviewer and a conclusion will be reached. The aim of this phase is to ensure that the studies are providing evidence which represent true statistical similarity and level of difference. The internal (design, conduct, and analyses) and external (abundance/diversity/activity, interventions, and outcomes) validity will be assessed by applying the five quality domains mentioned below and defined as low, high or uncertain and the results will be reported separately for each domain.

The assessment will be performed using check lists by one reviewer as a random (20%) subsample of the studies will be performed by second reviewer and the results will be compared with Kappa statistics. The inconsistencies (Kappa value less than 0.6) will be discussed and a third reviewer will be asked to judge in case no agreement could be reached. Records with the evaluation results and the reasons for judgment will be made for each article included at this stage.

Selection bias

Pre-treatment differences between the studied groups and in the baseline characteristics of the study will be assessed in the selection bias domain. The following elements will be assessed:

- **plot location** - low risk if the experimental plots of both the intervention and the control treatment are located in one field or other experimental unit with known history. High risk if the experimental units are located at different fields and there is no nformation about the history of the field.
- **comparator** - low risk if the comparison is *Bt* crop vs corresponding isogenic line. High risk if for the control treatment another variety is used.
- **randomisation** - the randomisation is the best way to avoid selection bias thus studies which are designed by any block or plot randomization method will be considered as low risk. High risk studies will occur if there is no or poor randomization.
- **replications** - low risk if there is at least three replicates per treatment. High risk if there is no replication.

Performance bias

Performance bias arises if the studied groups are influenced by factors different from the intervention, which may have an effect on the measured outcomes. In the field studies, such influences might come from:

- **plot size** - low risk if the plot size is properly defined in relation to the actual measurements; high risk if plot size is very small.
- **field management** - low risk if both control and intervention received the same agro-technical management including tillage, fertilizers, pesticides, irrigation, cultivation etc. High risk if there are differences in the management between the treatments.

Measurement bias

The way the measurement of the outcomes is done can influence the true effect estimation if the selected method is not accurate or can be influenced by the human subjectivity.

- **sampling of soil microorganisms** - the experimental procedures are crucial when assessing the abundance/diversity and/or activity of microorganisms. When the experiments are performed using standardized techniques or other recognized methods the risk of bias will be considered low. If the technique is not suitable for the examined questions, or is prone to human influence the risk of bias, will be considered high.

Attrition bias

Imbalance in the final set of selected studies will be assessed. In theory, an imbalance may occur if studies with the following properties are excluded:

- **sampling size** - low risk if the sample size is equal between the treatments. High risk if it differs due to loss of samples.
- **missing data** - low risk if the amount of data for the measured outcomes is equal for all the treatment. High risk if there is imbalance in the presented outcome data between the treatments.

Data extraction strategy

The aim of the data extraction stage is to retrieve information relevant to the design, performance and measured outcomes, which are intended to be used for the quantitative/qualitative synthesis and the analysis of the variability between studies. Details about the experimental sites and design will be extracted from the text in the sections describing the materials and methods of the study, as well as the description of other experimental procedures and statistical analysis. Numeric/binary/qualitative data for the measured outcomes and will be extracted from tables and figures in the result section of the study. The most direct original data are always selected; derived data are only selected if the datasets are restricted to these.

All data will be imported in standardised Excel tables. Each measurement of abundance/diversity/activity of the microorganisms as affected by the different treatment (e.g. *Bt* crop and comparator) will be included in the table as separate records containing all the defining variables. Data extraction will be performed by one review team member and checks will be performed by other review team members of a random 10% subset of the data. If necessary, discrepancies will be resolved by the involvement of a third reviewer.

Data to be extracted from the papers is presented in Table 2.

Data analysis

The extracted data will be used to synthesize the evidence provided by the individual studies about the effects of *Bt* crop cultivation to soil microorganisms, as well as to investigate the heterogeneity among the studies.

The data will include abundances as measured by different techniques and at different taxonomic levels, different measures for diversity and microbial activity as measured by different techniques. The included data will be quantitative, but will include categorical as well as binary data. Initially data of the same kind will analysed together, later data synthesis via meta-analysis will be performed. For this purpose data are likely to be transformed.

Assessment of statistical power of included studies

All data that meet the above requirements will be used in the analysis, irrespective of the statistical power of the individual study.

Measures of treatment effect

Depending on the studied measurements the abundance/diversity, activity can be measured per volume of soil (measured as volume or gram dry matter) or per plot. The reported values are usually the mean for the treatment and the calculation of the associated variance.

Dealing with missing data

If the measurements of either the treated plots or the control plots are missing then the study is not included in the analysis. In cases when for reporting the variance other values than standard deviation or standard error is used, e.g. t, F, p or z-values, an appropriate mathematical method will be used to calculate the pooled standard deviation, if appropriate. If this is not feasible, authors will be contacted to provide the missing data.

Synthesis

Quantitative synthesis

Quantitative synthesis will be performed to combine the magnitude of the effects from the individual studies. The analysis will include calculation of the pooled effect size for each study accompanied with the corresponding confidence intervals. The results from the analysis will be presented graphically in 'forest plots', where the estimated effect size with the confidence interval of each individual study will be plotted horizontally as the combined effect size and confidence interval will be plotted below them.

The complete dataset will be stored in the GRACE CADIMA database for open access after the finalisation of the review. For mixed effects modelling SAS PROC MIXED, PROC GLIMMIX or R ver. 3 [18] will be used.

A range of effect modifiers will be extracted from the selected papers and stored in the database (Table 2). These effect modifiers include comparator properties, pesticide treatment, insect pressure, experimental design, cropping system, crop rotation, tillage date, date of GM experimental cropping system establishment and soil type.

Assessment of heterogeneity

The heterogeneity across the studies that may influence the outcome will be assessed. In field studies, estimating the effects of *Bt* crop cultivation to soil microorganisms several sources of contextual heterogeneity may be expected:

Table 2 Information to be extracted from the papers qualifying for inclusion in the review and to be stored in the CADIMA database

Variable name	Definition
Geo-reference, longitude (field)	GPS coordinate WGS 84
Geo-reference, latitude (field)	GPS coordinate WGS 84
Location	Location of the experimental site
Crop	Name of the crop
GM Event	Name of the GM event
Treatment property	Type of *Bt* toxin(s) or comparator
Gene stacking	Information about stacked event , name of the stacked genes
Variety and cultivar	The commercial name of the variety and cultivar ID
Comparator properties	Information about the used comparator in relation with the *Bt* variety - isogenic or other.
Pesticide treatment(s)	Information about pesticide treatment , product name, active substance, amount, number, time and method for application.
Fertilizer use	Amount of NPK
Weather conditins	Mean annual temperature and precipitation
Experimental setup	Field; glasshouse; microcosmos; mesocosmos
Experimental design	Randomized complete blocks; Completely randomized; Multi location; Other. Kind of micro-mesocosmos
Plot size (field)	Calculation of the plot size (in square meters)
Plots number (field)	Number of plots per treatment
Cropping system (field)	Conventional; Reduced tillage; Conservation tillage etc.
Crop rotation (field)	Information about the history of the experimental field
Tillage date (field)	Date since last tillage event, including ploughing, harrowing, rotovation etc.
Date of cropping system establishment (field)	Date when the *Bt* crop was planted for first time
Seeding date (field)	Date of seeding within each growing season
Sampling date (field)	Date when the sampling was performed
Plant development stage	Stage at the point of sampling
Size (Micro-mesocosmos)	Size of the Experimental setup
Number of replicates (Micro-mesocosmos)	Number of replicates per treatment
Duration (Micro-mesocosmos)	Duration of the experiment
Temperature (Micro-mesocosmos)	Temperature at which the experiment was carried out
Other (Micro-mesocosmos)	Other informations related to experiments in Micro-mesocosmos
Data type	Of the value of the measured quantity, real, integer, binary, categorical, other
Method(s)	Short description of the applied method(s)
Microbial biomass	Measured biomass
Bacterial abundance	Number of bacteria, total and/or specific groups
Fungal abundance	Number of fungi, Total and/or specific groups
Microbial diversity	Taxonomic, genetic and/or functional diversity
Microbial activity	Specific activity measures, includes respiration, enzymatic activities, mineralisation etc.
Other microbial measurers	Other microbial measurements, including colonization of roots by mycorrhizal fungi, and root nodules
Sample depth (field)	Depth in the soil where the sample was taken
Sample amount	Amount of soil in one sample
Sampling location (field)	Location from which the samples are take, e.g. between rows, within rows, distance from roots
Extraction method	Used technique for extraction of microorganisms, the activity or DNA etc.
Sample size (field)	Sample size as reported by the author
Variability measure	STD; SEM; CLM; Variance

Table 2 Information to be extracted from the papers qualifying for inclusion in the review and to be stored in the CADIMA database *(Continued)*

Quantity	Quantified variability
Soil type	Soil type according to WRB classification
Other fixed and random effects	E.g. fertilization, fungicide use, additional factors and effects of the experimental design
Data origin	Table or figure from which the data originates
Data extraction	How data was extracted, e.g. as exact number from tables or scaled numbers from graphs
Statistical analysis	Description of the statistical methods for analysis of variance
Reference	Bibliographic reference code as found in the GRACE CADIMA database (Central Access Database for Impact Assessment of Crop Genetic Improvement Technologies)
Source of funding	Description of the funding source of the study
Authors affiliation	Type of institution to which the first author belongs
Comments	Any other information which may be relevant
Keywords	Keywords for finding the reference in a systematic review - {list of keywords}

Heterogeneity in studied abundances/diversities/activities The examined measurements will vary among the individual studies. They will even differ in whether abundances, diversity or activity are measured and all kinds of combinations will exist. The techniques used will also vary among the studies.

Heterogeneity in type of exposure A source of heterogeneity related to the type of exposure will be the variability of the GM plant species and the type of *Bt* toxin, i.e. the CRY event, which they produce. Among the most widely studied *Bt* crops are maize producing Cry1Ab (against lepidopteran pests) and Cry3Bb1 (against coleopteran pests), followed by Cry1Ac producing cotton, also protecting against lepidopteran pests.

Heterogeneity in methodology The main source of variability between the studies related with the methodology could be expected from differences in the experiment duration and time of sampling. Some authors report on short-term studies, others on one or two years study with one sampling at the end of the season, while others report multi-year studies with several sampling occasions in the beginning, in the middle and in the end of the season. Another source of variability might be that the effect of different Bt-cultivars differs. Considering the possible cumulative effect of the *Bt*-toxin in the soils, the measurements carried out in several subsequent years will be compared separately if corresponding data is found. In addition we will examine cumulative data sets in order to distinguish between cumulative effects of *Bt*-toxins and the cumulative effectseffect of the cultivation of *Bt* crops.

Heterogeneity in comparisons The experimental design of the studies can include one or combination of the following comparisons: *Bt* with non-*Bt* plots, neither of which received any insecticide treatments; unsprayed *Bt*-plots with non-*Bt* plots that received insecticides and *Bt* with non-*Bt* fields when both are subject to insecticide treatments.

To deal with the above mentioned contextual heterogeneity sub-group analysis will be performed if data suitable to calculate the effect size is found in minimum three distinct publications, which contain the same *Bt* crop producing and type of Cry toxin and the same kind of measurements. Analysis on the finest possible level will be made.

Other sources of heterogeneity which may have effect on the outcomes of the studies could come as a result of differences in the methodology such as plot size, sample size, experimental procedure and field management, or to be caused by the different factors of the environment or the experimental setup.

Statistical analysis of heterogeneity Heterogeneity will be addressed in conventional analyses of correlation, regression and mixed modelling ANOVA to reveal if the heterogeneities have any significant impact on the *Bt* crop effect estimates. E.g. if the studies can be grouped into soil type categories holding sufficient number of replicates, hypotheses about the effect of soil type on the outcome of effects can be elucidated. This will be one type of sensitivity analysis. In principle all the effects modifiers can be included in sensitivity analyses, i.e. do their inclusion or exclusion affect the assessment.

Sensitivity analysis

The degree to which the different sources of heterogeneity influence the results will be estimated.

The following factors will be considered:

- weight of the individual study;
- study quality;

- study funding.
- methodological differences

Assessment of publication bias

In the medical research it is known that "positive" results are more likely to be published [19]. Publication bias will be assessed using "funnel plot" analysis, where the effect size of an individual study will be plotted on the horizontal axis and the standard error or sample size on the vertical axis. The asymmetry in the funnel plot may indicate publication bias [20].

Review teams

The literature searches and data extraction will be conducted by teams at ABI and Aarhus University jointly, while the team at Aarhus University will be the main responsible for data analysis and synthesis. Furthermore, "Sweet Environmental Consultants" will provide internal quality assurance of the review process.

Appendix

List of studies used for the relevance check in the search exercise.

1. Title: Field Evaluation of Arbuscular Mycorrhizal Fungal Colonization in Bacillus thuringiensis Toxin-Expressing (Bt) and Non-Bt Maize

Author(s): Cheeke, Tanya E.; Cruzan, Mitchell B.; Rosenstiel, Todd N.

Source: APPLIED AND ENVIRONMENTAL MICROBIOLOGY Volume: 79 Issue: 13 Pages: 4078–4086 DOI: 10.1128/AEM.00702-13 Published: JUL 2013

2. Title: Increased microbial activity and nitrogen mineralization coupled to changes in microbial community structure in the rhizosphere of Bt corn

Author(s): Garcia-Villaraco Velasco, A.; Kowalchuk, G. A.; Gutierrez Manero, F. J.; et al.

Source: APPLIED SOIL ECOLOGY Volume: 68 Pages: 46–56 DOI: 10.1016/j.apsoil.2013.03.010 Published: JUN 2013

3. Title: Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties

Author(s): Dohrmann, Anja B.; Kueting, Meike; Juenemann, Sebastian; et al.

Source: ISME JOURNAL Volume: 7 Issue: 1 Pages: 37–49 DOI: 10.1038/ismej.2012.77 Published: JAN 2013

4. Title: Effect of Cry1Ab Protein on Rhizobacterial Communities of Bt-Maize over a Four-Year Cultivation Period

Author(s): Barriuso, Jorge; Valverde, Jose R.; Mellado, Rafael P.Source: PLOS ONE Volume: 7 Issue: 4 Article Number: e35481 DOI: 10.1371/journal.pone.0035481 Published: APR 30 2012

5. Title: Effect of vegetation of transgenic Bt rice lines and their straw amendment on soil enzymes, respiration, functional diversity and community structure of soil microorganisms under field conditions

Author(s): Fang, Hua; Dong, Bin; Yan, Hu; et al.

Source: JOURNAL OF ENVIRONMENTAL SCIENCES-CHINA Volume: 24 Issue: 7 Pages: 1259–1269 DOI: 10.1016/S1001-0742(11)60939-X Published: 2012

6. Title: Microbial properties, enzyme activities and the persistence of exogenous proteins in soil under consecutive cultivation of transgenic cottons (Gossypium hirsutum L.)

Author(s): Chen, Z. H.; Chen, L. J.; Zhang, Y. L.; et al.

Source: PLANT SOIL AND ENVIRONMENT Volume: 57 Issue: 2 Pages: 67–74 Published: 2011

7. Title: Bt corn plants and their straw have no apparent impact on soil microbial communities

Author(s): Tan, Fengxiao; Wang, Jianwu; Feng, Yuanjiao; et al.

Source: PLANT AND SOIL Volume: 329 Issue: 1–2 Pages: 349–364 DOI: 10.1007/s11104-009-0163-2 Published: APR 2010

8. Title: Impact of coleopteran targeting toxin (Cry3Bb1) of Bt corn on microbially mediated decomposition

Author(s): Lawhorn, C. Nicole; Neher, Deborah A.; Dively, Galen P.

Source: APPLIED SOIL ECOLOGY Volume: 41 Issue: 3 Pages: 364–368 DOI: 10.1016/j.apsoil.2008.12.003 Published: MAR 2009

9. Title: Transgenic Bt-Cotton affects enzyme activity and nutrient availability in a sub-tropical inceptisol

Author(s): Sarkar, B.; Patra, A. K.; Purakayastha, T. J.

Source: JOURNAL OF AGRONOMY AND CROP SCIENCE Volume: 194 Issue: 4 Pages: 289–296 DOI: 10.1111/j.1439-037X.2008.00312.x Published: AUG 2008

10. Title: Neither transgenic Bt maize (MON863) nor tefluthrin insecticide adversely affect soil microbial activity or biomass: A 3-year field analysis

Author(s): Devare, M.; Londono-R, L. M.; Thies, J. E.

Source: SOIL BIOLOGY & BIOCHEMISTRY Volume: 39 Issue: 8 Pages: 2038-2047 DOI: 10.1016/j.soilbio.2007.03.004 Published: AUG 2007

11. Title: Transgenic Bt cotton has no apparent effect on enzymatic activities or functional diversity of microbial communities in rhizosphere soil

Author(s): Shen, Ren Fang; Cai, Hong; Gong, Wan He

Source: PLANT AND SOIL Volume: 285 Issue: 1–2 Pages: 149–159 DOI: 10.1007/s11104-006-9000-z Published: JUL 2006

12. Title: Changes of Bt toxin in the rhizosphere of transgenic Bt cotton and its influence on soil functional bacteria

Author(s): Rui, YK; Yi, GX; Zhao, J; et al.

Source: WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY Volume: 21 Issue: 6–7 Pages: 1279–1284 DOI: 10.1007/s11274-005-2303-z Published: OCT 2005

13. Title: Differences in yields, residue composition and N mineralization dynamics of Bt and non-Bt maize

Author(s): Mungai, NW; Motavalli, PP; Nelson, KA; et al.

Source: NUTRIENT CYCLING IN AGROECOSYSTEMS Volume: 73 Issue: 1 Pages: 101–109 DOI: 10.1007/s10705-005-8850-8 Published: SEP 2005

14. Title: Bacterial diversity in rhizospheres of nontransgenic and transgenic corn

Author(s): Fang, M; Kremer, RJ; Motavalli, PP; et al.

Source: APPLIED AND ENVIRONMENTAL MICROBIOLOGY Volume: 71 Issue: 7 Pages: 4132–4136 DOI: 10.1128/AEM.71.7.4132-4136.2005 Published: JUL 2005

15. Title: Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere

Author(s): Baumgarte, S; Tebbe, CC

Source: MOLECULAR ECOLOGY Volume: 14 Issue: 8 Pages: 2539–2551 DOI: 10.1111/j.1365-294X.2005.02592.x Published: JUL 2005

16. Title: Changes in soil microbial community structure associated with two types of genetically engineered plants analyzing by PLFA

Author(s): Xue, K; Luo, HF; Qi, HY; et al.

Source: JOURNAL OF ENVIRONMENTAL SCIENCES-CHINA Volume: 17 Issue: 1 Pages: 130–134 Published: 2005

17. Title: Soil microbial communities associated with Bt and non-Bt corn in three soils

Author(s): Blackwood, CB; Buyer, JS

Conference: Symposium on Environmental Impact of Transgenic Crops on Soil Biological Processes and Functions Location: Minneapolis, MN Date: 2002

Source: JOURNAL OF ENVIRONMENTAL QUALITY Volume: 33 Issue: 3 Pages: 832–836 Published: MAY-JUN 2004

18. Title: Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the Bacillus thuringiensis var tenebrionis endotoxin

Author(s): Donegan, KK; Schaller, DL; Stone, JK; et al.

Source: TRANSGENIC RESEARCH Volume: 5 Issue: 1 Pages: 25–35 DOI: 10.1007/BF01979919 Published: JAN 1996

19. Title: Changes in levels, species and dna fingerprints of soil-microorganisms associated with cotton expressing the bacillus-thuringiensis var kurstaki endotoxin

Author(s): DONEGAN, KK; PALM, CJ; FIELAND, VJ; et al.

Source: APPLIED SOIL ECOLOGY Volume: 2 Issue: 2 Pages: 111–124 DOI: 10.1016/0929-1393(94)00043-7 Published: JUN 1995

Additional file

Additional file 1: Details on the calculation of Kappa (K).

Competing interests

None of the members of the review team has any financial interest in the outcome of the systematic review nor are they affiliated with any religion or organisation that have expressed a certain position to GMO.

Authors' contributions

KK, PHK, JBS, CFD, NBH prepared this review protocol. NBH made the data extraction of the pilot literature search. PHK and JBS provided study quality criteria and GRACE project management. All authors read and approved the final manuscript.

Acknowledgements

This work is funded within EU-FP7 project GRACE, Grant Agreement KBBE-2011-6-311957. We are grateful for the support and methodological guidance provided by the management and members of GRACE consortium. Michael Urbanczyk's help with the Word-processor was very useful.

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Received: 25 February 2014 Accepted: 13 May 2014
Published: 9 June 2014

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doi:10.1186/2047-2382-3-11
Cite this article as: Kostov *et al.*: Are soil microbial endpoints changed by Bt crops compared with conventional crops? A systematic review protocol. *Environmental Evidence* 2014 **3**:11.