Electronic supplementary material for

Assessing life cycle impacts from changes in agricultural practices of crop production

Methodological description and case study of microbial phosphate inoculant

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Environmental life cycle assessment of US corn produced with microbial phosphate inoculant

Final version

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Preface

This study assesses the environmental consequences of introducing a yield-enhancing microbial inoculant in US corn production. The study is based on life cycle assessment (LCA).

The study has been performed in accordance with the ISO standards for LCA (14040 and 14044) and the modeling of environmental impacts has been performed with the biogeochemical model DayCent and the LCA software tool SimaPro 8 (version 8.0.5.13) using the impact assessment method called CML-IA baseline (version 3.01).

This study has been under external critical review conducted by the following panel.

- Prof. Michael Hauschild, Technical University of Denmark (chair of the panel)
- Dr. Lorie Hamelin, senior researcher, Institut National des Sciences Appliquées (INSA), Toulouse, France
- Dr. Nuala Fitton, research fellow, University of Aberdeen, United Kingdom

The review statement is available as Appendix H.

Minor changes have been made to this version of the report in March 2019. These changes have been approved by the review panel. They have no implications for the conclusions of the study. In addition, the inoculant dose was corrected in January 2020 with minor implications for results. This has been approved by the chairman of the review panel.

Acknowledgement

The authors wish to thank Birger Stjernholm Madsen (Novozymes) for assistance with the uncertainty analysis in the JMP software tool.

Summary

A brief none-exhaustive summary of the present LCA report is provided below.

Goal and Scope

The purpose of this environmental life cycle assessment (LCA) is to assess the consequences for the environmental impacts from US corn production when introducing a seed treatment (inoculant) containing spores of the naturally occurring soil fungus called *Penicillium bilaiae* (*P.b.*).

The functional unit of the study is defined as 1 metric tonne (Mg) of dried corn kernels (14% moisture).

Impact Categories and Methods

The study addresses the following impact categories of which the first (global warming) receives the main attention.

1) Global warming (GWP100)
2) Acidification
3) Eutrophication (nutrient enrichment)
4) Photochemical ozone formation (smog formation)
5) Fossil energy resources
6) Land occupation

The study applies ‘consequential LCA’, which is characterized by the use of marginal data and the use of so-called ‘system expansion’ in case of co-products/multi-output processes.

Inventory Analysis

The study focuses on continuous corn (corn after corn) and corn in a corn/soybean rotation (corn after soybeans) in Minnesota and North Dakota. The inventory analysis is based on yield increase data from the scientific literature, biogeochemical modeling, LCA data from the ecoinvent database, and a previous study, which modeled the production of the *P.b.* inoculant.

Impact Assessment

The introduction of the *P.b.* inoculant is found to reduce the impact of corn production in all investigated categories, particularly for global warming and eutrophication where reductions of 9-15% are observed (base case results for Minnesota and North Dakota). More modest improvements (2-4%) are estimated for the remaining impact categories. In terms of global warming, the impact of producing one Mg corn was reduced by 34-40 kg CO_2e (base case results) when applying the *P.b.* inoculant.

Sensitivity and uncertainty analyses

The impact of different time perspectives and a different approach for estimating changes in CO_2 emissions from changes in soil organic carbon (SOC) as well as potential impacts from changes in the root fraction of corn with *Penicillium bilaiae* were investigated in the sensitivity analyses. In addition, the potential indirect land use change (ILUC) from increasing yields on existing cropland was considered in relation to GHG emissions. Results of all sensitivity analysis were between 30 and 50 kg CO_2e saved per Mg of corn produced with *Penicillium bilaiae*.

A quantitative uncertainty analysis was conducted for the global warming results (base case results only). The relative 95% confidence intervals Minnesota and North Dakota (regardless of crop rotation) were respectively -28% / +25% and -43% / +41%. The dominating source of uncertainty was the uncertainty related to the yield increases obtained with the *P.b.* inoculant.

Conclusions and perspectives

The study suggests that the use of *P.b.* in US corn production provides significant environmental benefits with no trade-offs. By extrapolation of the base case results for Minnesota and North Dakota, it is estimated that the *P.b.* inoculant could reduce GHG emissions by 3.9 million Mg CO_2e if applied on all US corn fields.

Recommendations

Additional research in *P. bilaiae's* potential impact on root fraction and harvest moisture is encouraged as this could have a substantial impact on LCA results. Besides, the LCA could be improved by fine-tuning the consistency between the biogeochemical modeling in DayCent and the remaining modeling in the LCA software tool (SimaPro). Finally, the LCA indicates that it is recommendable from an environmental point of view to rather apply *P.b.* on too many fields than too few. The reason is that the environmental impacts associated with the use of *P.b.* on non-responsive fields[1] are compensated many times by the reduced impacts from responsive fields.

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[1] Fields where the *P.b.* inoculant does not impact the yield

List of abbreviations

GHG	Greenhouse gas
ILUC	Indirect land use change
LCA	Life cycle assessment
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
LHV	Lower heating value
Mg	Megagram (equal to one metric ton or 1,000 kg)
MJ	Megajoule
P.b.	*Penicillium bilaiae*
RSB	Roundtable on Sustainable Biomaterials
SOC	Soil organic carbon

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1 Introduction

The growing demand for agricultural products is projected to continue in the coming decades (Alexandratos and Bruinsma, 2012). This will require the effective management of our natural resources such as fertile land and phosphorus to mitigate the impact of agriculture-based greenhouse gas (GHG) emissions (Robertson et al., 2000) and limit nutrient losses to aquatic ecosystems (Carpenter et al. 1998). These issues illustrate the need to develop more sustainable agricultural practices that better optimize the use of resources and limit emissions per unit of agricultural output. Follett et al. (2011) presented a number of agricultural practices (e.g. manure addition to cropland and no or reduced tillage) that increased soil organic carbon (SOC) stocks and thereby decreased the atmospheric CO_2 concentration, mitigating climate change. Clay et al. (2012) utilized a more product-oriented approach and demonstrated how carbon sequestration in US croplands can have significant implications for the LCA of ethanol produced from corn. Similarly, Popp et al. (2011) developed a methodology for crop production LCA at county level in Arkansas, and Mamani-Pati et al. (2010) used LCA to assess the energy efficiency of different corn production systems in South Dakota. While these studies focused on a single impact category (either GHG emissions or energy efficiency), the LCA methodology (ISO, 2006a; ISO, 2006b) allows for inclusion of a broader range of resource and impact categories[2]. This enables the investigation of potential environmental trade-offs in relation to a change, e.g. in agricultural practices. For instance, one of the obvious options for improving land use efficiency is to increase crop yields per hectare. If this increase is achieved by additional fertilizer use, there may however be a trade-off in nitrogen losses to the aquatic environment and/or N_2O emissions to the atmosphere. This illustrates the complexity of evaluating the impact on environmental quality when implementing a change in agricultural practices – but also the strength of the LCA methodology. In this study, we use LCA to study the environmental impacts of a different option for improving yields in crop production.

Based on several hundred field trials, Leggett et al. (2015) documented how the use of the fungus *Penicillium bilaiae* Chalabuda can increase yields per hectare in US corn production. The fungus solubilizes phosphorus by secretion of organic acids (Cunningham and Kuiack 1992). This effect can be utilized in crop production by seed treatment and subsequent colonization of crop roots (Leggett et al. 2015). Besides solubilization of mineral phosphorus in close proximity to the roots, some studies suggest that *P. bilaiae* increases root length as well as root hair abundance (Gulden and Vessey 2000, Vessey & Heisinger 2001) thereby generating improved access to moisture and nutrients. These mechanisms are likely the main drivers of the yield increases documented by Leggett et al. (2015).

P. bilaiae is marketed under the name JumpStart® as a soluble powder consisting of fungal spores and various formulants. *P. bilaiae* is also sold as an integrated part of the seed inoculant called Acceleron® B-300 SAT. The inoculant is applied to the corn seeds prior to seeding.

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[2] In fact, ISO (2006b) not only allows but calls for a broader range of categories stating that *"The selection of impact categories shall reflect a comprehensive set of environmental issues related to the product system being studied, taking the goal and scope into consideration."*

2 Goal and Scope

This chapter gives a description of the objectives and the frames of the study.

2.1 Goal definition

The objective of this study was to estimate the environmental consequences of introducing *P. bilaiae* in US corn production.

2.1.1 Intended application

The final version of the LCA is intended to be used for multiple purposes. Results will be used in calculations to assess the overall GHG benefits of Novozymes' product portfolio. Results will also be used to support environmental claims related to the use of *P. bilaiae* in crop production. Furthermore, the study may also be used in relevant international fora and public policy processes that seek to highlight and support the development and deployment of better agricultural practices. Finally, the study is also intended to form the basis for an article in the peer-reviewed literature.

2.1.2 Reasons for carrying out the study

The study is carried out to make quantitative estimates of the environmental implications of introducing *P. bilaiae* in US corn production.

2.1.3 Intended audience

The study is intended for bilateral use with a number of relevant stakeholders (c.f. 'Intended application') and potentially as supplementary material for scientific publication.

2.1.4 Comparative assertion

The study considers a microbial inoculant with the ability to increase agricultural yields. The study does not make any comparison to other products that also have a yield-increasing ability. In that sense, the study does not represent a comparative assertion.

The study does however compare different options for using the increased yield obtained with *P. bilaiae* and the implications for the global warming impact category.

2.1.5 Data requirements

The study requires data on corn production, nitrogen and carbon flows at the field level, and production of the *P.b.* inoculant.

2.1.6 Limitations

Yield increases obtained with *P. bilaiae* can differ from field to field and the study addresses the average response. This means that the results are not necessarily applicable to any one corn field. Instead, the study seeks to give a general picture of the environmental consequences of using the *P.b.* inoculant on corn within the specified geographical scope.

2.1.7 Critical review

The study has been subject to a critical review in accordance with the ISO standards for LCA (ISO 2006a, ISO 2006b). The review has been conducted by the following panel.

- Prof. Michael Hauschild, Technical University of Denmark (chair of the panel)
- Dr. Lorie Hamelin, senior researcher, Institut National des Sciences Appliquées (INSA), Toulouse, France
- Dr. Nuala Fitton, research fellow, University of Aberdeen, United Kingdom

2.1.8 Type and format of report

The present report is a technical document structured on the basis of the guidelines given in the ISO standards for LCA (ISO 2006a, ISO 2006b).

2.2 *Scope definition*

This section elaborates on system characteristics, the functional unit, methodology, impact categories, etc.

2.2.1 Product systems studied

The study considers corn production with *P. bilaiae* in the two following crop rotations.

- Continuous corn rotation (corn after corn)
- Corn/soybean rotation (corn after soybeans)

Conventional corn production without *P. bilaiae* is considered as the reference system. A more detailed description of the corn production systems appears later in the report.

2.2.2 Geographical scope

The study focuses on corn production in Minnesota and North Dakota in the USA. These two states were selected for two reasons. First, they were among the four states with the highest number of large plot field trials in Leggett et al. (2015). Second, required site data was available for DayCent modeling.

While Minnesota and North Dakota are neighboring states, their regional contributions towards US corn production is notably different. Southern Minnesota is considered part of the ‘Corn Belt’ and the state is among the top producers in the country, providing more than 10% of the annual harvest. In contrast, North Dakota, with its much drier climate, is not considered to be part of the 'Corn belt' but still has a large area dedicated towards corn production. However, the overall production level is much lower as reported by Leggett et al. (2015).

Despite the differences, the present report shows that the LCA results (per functional unit) are quite similar for the two states. One of the reasons is that relative yield increases (observed with *P. bilaiae*) are more or less the same for Minnesota and North Dakota (4.1% and 4.4%, respectively). As will later be discussed in detail, the yield increases drive the dominating effects studied in the LCA (changes in field nutrient flows and displacement of crop production elsewhere). As these mechanisms are quite generic, it has been considered reasonable to make a crude extrapolation to the entire US based upon the LCA results for Minnesota and North Dakota.

2.2.3 Temporal scope

The temporal scope is ‘now’. The *P.b.* inoculant exists and the study relies on field trial data from recent years (Leggett et al. 2015). Meanwhile, different time perspectives are explored in relation to the modeling of CO_2 emissions from changes in soil organic carbon (further explained later in the report).

2.2.4 The functional unit

The study seeks to answer the following question: How are the environmental impacts from corn production in Minnesota and North Dakota affected when a microbial seed treatment containing spores of *Penicillium bilaiae* is introduced (while keeping all other inputs per hectare equal)? The functional unit of the study (to which the changes in impacts will be related) is one metric tonne (Mg) of dried corn kernels (14% moisture).

2.2.5 The system boundaries and cut-off criteria

The systems studied in the present LCA (including their boundaries) are further discussed in the next section and the cut-off criteria are defined as follows: Omitted aspects must generally be of low importance for the end results (less than five percent for omitted aspects in total) and omitted aspects should not favor the *P.b.* inoculant (conservative approach) unless the impact on end results is less than one percent. As for the few omissions made in the study, it has been assessed (case by case) if the potential impact on results would be in conflict with these quantitative cut-off criteria.

2.2.6 Methodology

A comprehensive description of the LCA methodology can be found in the ISO standards for LCA (ISO 2006a and ISO 2006b) and in Wenzel et al. (1997). It is beyond the scope of the present report to give an exhaustive description of the entire methodology but the present section will focus on methodological aspects, which are particularly relevant for a consequential assessment of technologies and agricultural practices, which impact crop yields, e.g. the introduction of a yield enhancing inoculant.

The general approach applied in the present study (and in consequential LCA as such) is to compare the studied system to a reference system, which provides the same functional output, and then analyze the difference in environmental impacts seen in relation to the functional unit. Here, the studied system is corn production with the use of *P. bilaiae* and the reference system is corn production *without* the use of *P. bilaiae*. Hence, the goal is to estimate the changes in environmental impacts which occur when *P. bilaiae* is introduced in conventional corn production.

Any new technology or practice, which has an impact on crop yields is likely to cause environmental impacts (positive or negative) in three different ways, which are summarized here.

Firstly, there may be upstream impacts. For instance, if shifting to ‘no till’, there is a reduced need for upstream fuel production and, if changing the amount or type of fertilizer or pesticides applied, this will also have an upstream impact. In the present study, the upstream impacts are related to the production of the microbial inoculant.

Secondly, the implementation of a new technology or practice with impacts on crop yields will also have an impact on the direct emissions from the crop field. The reason is that changes in yield will automatically be associated with changes in nutrient flows, which in turn impact emissions to the atmosphere (e.g. nitrous oxide) and nutrient losses to the aquatic environment (e.g. nitrate). Such impacts can be roughly estimated via simple mass balance considerations or they can be analyzed via more sophisticated biogeochemical models (such as the DayCent model applied in the present study). The impacts related directly to changes in the soil of the cropland will here be referred to as ‘the field effect’.

Thirdly, the change in output from the area where a new technology or agricultural practice is applied will have market-mediated effects elsewhere. If the yield per hectare is reduced (e.g. due to a shift to less intensive practices), it will likely stimulate increased production in a different location and, if the yield per hectare is increased, the additional output will likely displace other (less competitive[3]) production (Kløverpris et al. 2008). The additional yield can be considered a special case of a co-product and thereby handled by ‘system expansion’. This means that the product system is expanded to include the additional functions of the co-product (ISO 2006b), which in this case would be the extra (or the reduced) yield. The function of additional yield would be displacement of crop production elsewhere. For example, if one hectare produces an additional Mg of barley, this will displace an equivalent amount of crop production elsewhere. This approach is well in line with system expansion applied in other agricultural LCAs, e.g. the approach for handling feed protein, which is co-produced with bioethanol from corn. In this example, the feed co-product is assumed to displace a corresponding amount of corn and soybean meal (see e.g. Wang et al. 2012). In the present study where the displacement effect is caused by a change in output from a specific area of cropland, the resulting change in environmental impacts will be referred to as ‘the yield effect’.

In summary, the present study seeks to analyze the environmental impact of the three following effects related to the use of *P. bilaiae* in US corn production.

1.	Upstream effects	Impacts from the production of the inoculant
2.	The field effect	Change in direct emissions from the cropland where *P.b.* is applied (studied by use of biogeochemical modeling)
3.	The yield effect	Impacts avoided through displacement of crop production elsewhere (studied by use of system expansion)

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[3] In the base case analysis of the present study, ‘less competitive’ production is implicitly assumed to be ‘standard corn production’ without the use of *P. bilaiae* within the same state (see end of Section 2.2.6.1). This is in line with system expansion applied in other LCAs mentioned in this study, e.g. the study by Wang et al. (2012). While the approach is common practice, ‘standard corn’ might not be the ‘less competitive’ crop production ultimately affected by yield increases on existing corn fields. It might not even be corn. Crops are usually traded based on their nutritional characteristics, most importantly feed energy and protein. Hence, they can replace each other in the market place. It is these mechanisms that have led to the discussion of ‘indirect land use change’, which will later be discussed in the present report.

2.2.6.1 Continuous corn

Fig. 1 outlines a conceptual sketch of the reference system for continuous corn as well as the corn production system with the *P.b.* inoculant[4].

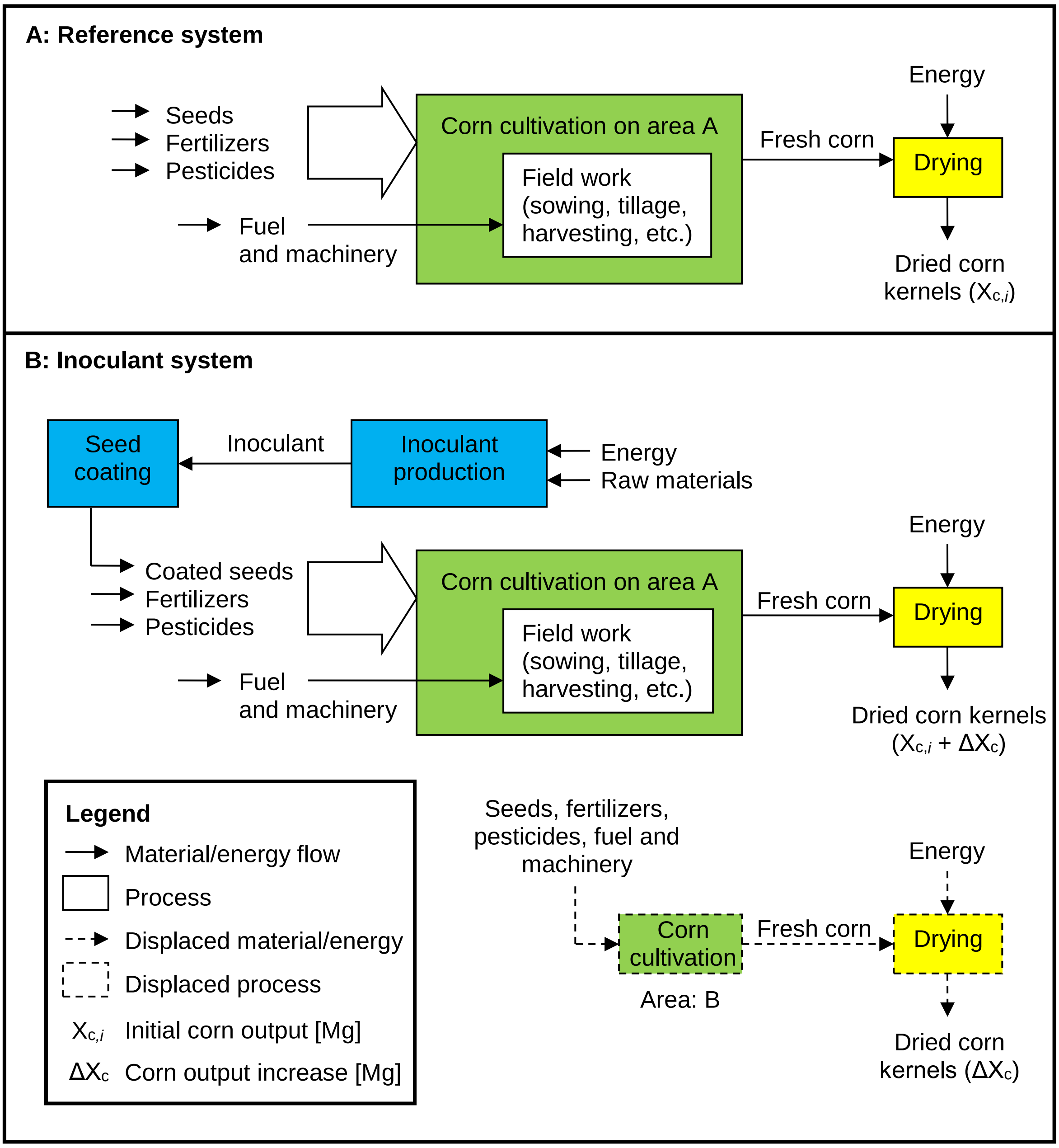

Fig. 1: Overview of reference system (A) and inoculant system (B) for continuous corn production (corn after corn)

The reference system represents standard crop production where agricultural inputs are applied to the field and the crop is harvested and dried to give a certain output of corn from the area A. Once the impacts from the system are estimated, they can be related to the functional unit (standard LCA approach). The inoculant system is slightly more

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[4] Note that Fig. 1 represents the conceptual modeling approach applied in the LCA. It does not seek to describe the field trials upon which the LCA rests. For a brief description of the field trials, please see Section 3.1 and, for a detailed description, please refer to Leggett et al. (2015).

complicated. First of all, it contains production of the *P.b.* inoculant (representing the upstream effect). Secondly, the direct emissions from the field are different than in the reference system (causing the field effect – not shown in the figure) and, thirdly, the inoculant system has a higher output of corn from the area A than the reference system ($X_{c,i} + \Delta X_c$) so it has been expanded to include displacement of corn production elsewhere (the yield effect). The system expansion ensures that the two systems provide the same output ($X_{c,i}$) and this 'system equivalence' enables a direct comparison of the two systems.

Fig. 1 is supplemented here with a further description of the symbols used.

- A is the area cultivated in the reference system
- $X_{c,i}$ is the initial (*i*) output of corn (c) from the area A
- ΔX_c is the increase (Δ) in output of corn (c) from the area A when *P.b.* is used
- B is the area of corn production displaced when *P.b.* is used

Based upon the symbols above, the following definitions are derived for further use in the report.

- Initial corn yield in the reference system: $Y_{c,i} = X_{c,i} / A$
- Yield increase with *P. bilaiae*: $\Delta Y_c = \Delta X_c / A$

Note that the relative reduction in area can be calculated as B divided by A (area displaced divided by the area in the reference system).

In the base case of the present study, it is assumed that the corn production displaced is conventional corn production (same as the reference system). This is in line with the standard system expansion procedure applied in many other agricultural LCAs, e.g. the example of bioethanol from corn mentioned above. Meanwhile, the yield effect from the application of *P. bilaiae* (i.e. the displacement of crop production elsewhere) could also be viewed in the light of recent years' discussions within the LCA community about so-called indirect land use change (ILUC). This topic will be handled later in the report as part of the sensitivity analyses.

When the corn production displaced is assumed to have the same yield as in the reference system (base case assumption), the area B (Fig. 1) can be calculated as follows.

- $B = \Delta X_c / Y_{c,i}$ (output displaced divided by yield in displaced system)

ΔX_c can be expressed as $\Delta Y_c \cdot A$ (see definition of ΔY_c above). When combined with the expression of B above, this gives:

- $B = (\Delta Y_c \cdot A) / Y_{c,i}$

The relative change in area (B / A) can thereby be expressed as follows.

- $B / A = \Delta Y_c / Y_{c,i}$

This formula is later used in the section concerning indirect land use change (ILUC).

2.2.6.2 Corn in a corn/soybean rotation

The corn-soybean rotation is a more complex system than continuous corn as it is an integrated system producing two functional outputs (corn and soybeans), although not at the same time. When applying *P. bilaiae* to corn, the yield goes up for corn but remains constant for soybeans. At the same time, field emissions in corn production are affected (as in the continuous corn system) but there is a spill-over effect to the field emissions from soybean production because the production of the two crops occurs on the same piece of land. To capture the entire field effect from introducing *P. bilaiae* on corn in a corn/soybean rotation, the systems outlined in Fig. 2 will be compared[5].

Fig. 2 is supplemented here with a further description of the symbols that have not already been described in relation to Fig. 1.

- $X_{s,i}$ is the initial (*i*) output of soybeans (s) from the area A

Note that both systems in Fig. 2 have the same output ($X_{c,i}$ + $X_{s,i}$) produced over the length of one full crop rotation, i.e. two years. Note also that the output from the soybean cultivation in year 2 is the same in the reference system and the inoculant system. The inputs to the soybean cultivation (fertilizers, pesticides, etc.) are also the same in the two systems. As consequential LCA is focused on changes (relative to a reference system), the inputs to soybean production and the output from it cancel out when calculating the difference between the two systems. Meanwhile, the emissions coming from the field (throughout the full crop rotation) will not necessarily be the same in the two systems (due to the inoculant introduced in corn production). The potential difference in field emissions can be estimated through biogeochemical modeling and thereby the field effect can be related to the functional unit, i.e. the production of 1 Mg of corn (the crop on which the cause of the change is applied). Note that the soybean production is only included in the analysis to account for the full field effect from introducing the *P.b.* inoculant in corn production.

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[5] Note that Fig. 2 represents the conceptual modeling approach applied in the LCA. It does not seek to describe the field trials upon which the LCA rests. For a brief description of the field trials, see Section 3.1 and, for a detailed description, see Leggett et al. (2015).

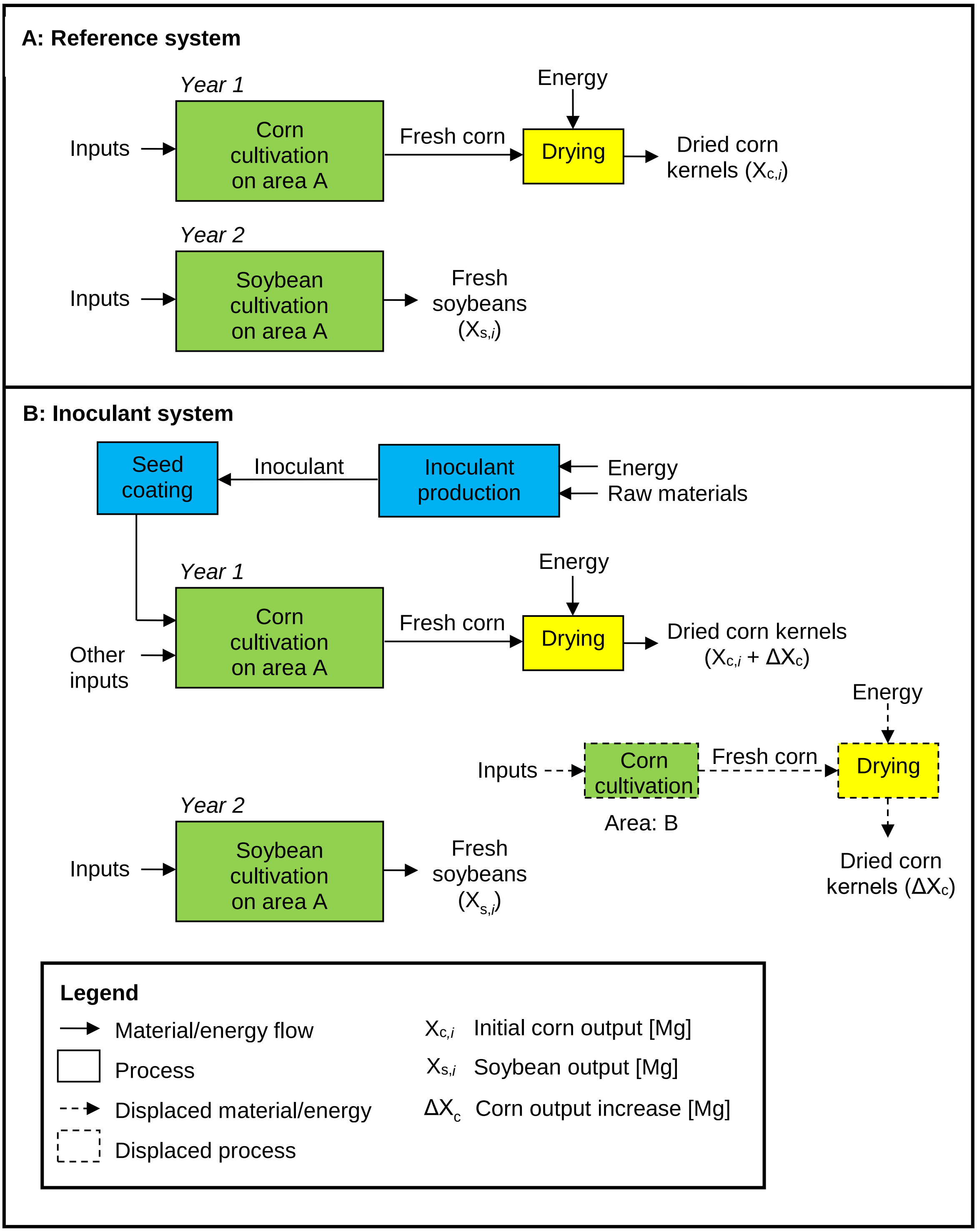

Fig. 2: Overview of reference system (A) and inoculant system (B) for corn production in a corn/soybean rotation (corn after soybeans)

2.2.6.3 Biogeochemical modeling with DayCent

The biogeochemical model DayCent (Del Grosso et al., 2009; Del Grosso et al., 2002) was applied to estimate the field effect. DayCent simulates nutrient flows, soil carbon, crop growth, and trace gas emissions in cropping systems. The model is a daily time step version of the CENTURY model, which simulates crop biomass production, C and N dynamics in the soil and trace gas emissions between the soil and atmosphere. The model is process-based and includes routines to estimate soil water and temperature by layer, crop growth and development. In DayCent, the elemental phosphorous is considered to be in a dynamic equilibrium between portions sorbed to the soil and that which is labile (Lewis and McGechan, 2002). Phosphorous stress can occur during plant growth as uptake is regulated by the amount of P in the labile fraction. Upper and lower boundaries of crop phosphorous requirements, which are set for both root and shoot fractions, constrain P uptake. Phosphorous can be lost from the system through soil erosion of the sorbed P or through leaching of the labile fraction. The model has been validated against data at a number of experimental sites in Canada and the United States (Grant et al., 2016; Congreves et al., 2015; Smith et al., 2012; Del Grosso et al., 2002, 2001), used to estimate the potential to mitigate GHG emissions worldwide (Del Grosso et al., 2009), and used to simulate bioenergy crop systems to develop an LCA of GHG emissions (Andler et al., 2007).

Using the DayCent model, corn after corn (continuous corn) and corn after soybean (a corn-soybean rotation) were simulated in Minnesota and North Dakota with and without the use of *P. bilaiae* over a 40-year period. The objective of this modeling approach was to provide estimates of net GHG emissions and C and N for average conditions across the experimental trials. Thus, inputs of N and P were based on values reported in Table 2 and yields were calibrated to match the field trials in Minnesota and North Dakota reported by Leggett et al. (2015)[6]. The default labile P fraction[7] in the model was set to 0.2 for the control treatments (no inoculant) based on site calibrations to ensure that residual levels of P were limited and this value was increased and calibrated on a site basis to match measured yield response when the *P. bilaiae* inoculant was applied. Climate data for Minnesota and North Dakota were obtained from the National Climate Data Centre[8]. It is generally thought that, to capture SOC changes induced from management, a period of 20 years is the very minimum time frame for which to observe these changes (Broch et al., 2013) with other studies suggesting an even longer duration may be necessary (McConkey et al., 2007). A 40-year modeling period was decided upon in order to ensure that changes in SOC dynamics would be well characterized and that other nutrient and trace gas losses, that are very highly correlated with inter-annual climate variability, were considered. A loam textured Mollisol was utilized as this represents the most predominant texture and soil order in the two investigated states. Following recommended DayCent modeling practice guidelines (Del Grosso et al., 2011), simulations for each location were initialized using a 2000-year spin-up in order to derive the respective carbon pool fractions (active, intermediate, and slow) under native vegetative conditions. This was then followed up with a short generalized regional cropping history of breaking the native vegetation into agriculture. Simplified historical rotations with low productivity and high intensity were assumed for this initial period to represent a general loss of native SOC stock towards a new equilibrium. These carbon pool fractions were then utilized in the 40-year simulation period to ensure that carbon pool dynamics were regionally representative. Initial soil carbon in all scenarios was 57.4 Mg ha^{-1}.

For all of the DayCent results except changes in SOC, we computed averages from the 40-year modeling period to derive representative results (see Table 5 and Table 6). For SOC, a different approach was taken due to the time dependency of this parameter. In the long

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[6] The yields applied in the DayCent modeling appear in Table 2.

[7] Parameter:<pslsrb> fraction of mineral P that is labile

[8] http://www.ncdc.noaa.gov/oa/climate/climatedata.htmls

term, changes in SOC will be insignificant[9] whereas they can be substantial in the short term. In the base case, the average annual change in SOC over the first 20 years was calculated and then converted to corresponding CO_2 emissions[10]. To smooth out differences in SOC between the reference system and the *P. bilaiae* system, due to inter-annual variations in weather, an exponential curve fitting approach was used (VandenBygaart et al. 2008). The 20 year annualization approach is also applied in the tier 1 approach in the IPCC guidelines for national GHG inventories (IPCC 2006). While the IPCC guidelines do not represent an LCA method, the 20 year annualization approach is also applied in the life cycle GHG accounting method in the European Renewable Energy Directive (EC 2009), and it has been applied in other LCA studies, e.g. Knudsen et al. (2010) and Hamelin et al. (2012). Meanwhile, there is currently no well-defined procedure for how to account for SOC changes in life cycle assessments (Goglio et al. 2015). Alternative time perspectives as well as a different methodological approaches were therefore explored as part of the sensitivity analyses.

2.2.7 Impact categories

The resource and environmental impact categories considered in the study are shown in Table 1. Global warming is the impact category which will receive the main attention.

Table 1: Overview of resource and environmental impact categories considered in the present study (descriptions based on Wenzel et al., 1997)

Category	Description of resources and emissions covered	Indicator
Global warming (GWP100)	Emissions contributing to climate change (100-year perspective)	CO_2 equivalents
Acidification	Emissions attacking the leaves of plants and contributing to acidification of soils and shallow freshwaters.	SO_2 equivalents
Eutrophication (nutrient enrichment)	Nutrient emissions contributing to potential algal bloom and oxygen depletion in aquatic ecosystems and species change in terrestrial ecosystems.	PO_4^{3-} equivalents
Photochemical ozone formation (smog formation)	Volatile organic compounds potentially leading to ozone formation during atmospheric degradation under the presence of nitrogen oxides with adverse effects on natural vegetation, agricultural production, and human health.	C_2H_4 equivalents
Fossil energy resources	Fossil energy resources measured as lower heating value, LHV (the energy released from combustion of a fuel excl. the heat required for vaporisation of the water generated during combustion)	MJ LHV†
Land occupation‡	Occupation of agricultural/forested land (time and area)	m^2y

† Mega Joule Lower Heating Value

‡ Added (by the authors) to the categories selected from the CML IA baseline method. In practice (in the present study), it is almost entirely arable land occupation but the category also covers occupation of forest, grassland, pasture/meadow, and permanent crop.

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[9] Both systems (reference and inoculant) will move towards their own equilibrium state in terms of SOC. When the systems approach each their own equilibrium, the difference in SOC between the systems will level out and become (almost) constant. In the long term, when the difference in SOC is amortized over a longer and longer time horizon, the change between the two systems will become insignificant.

[10] Conversion between soil carbon (DayCent results) and CO_2 (LCA results) were based on stoichiometry so that one kg of organic C (12 g/mol) would correspond to 3.67 kg CO_2 (44 g/mol) Hence, a decrease in SOC was assumed to occur through oxidation of organic C and an increase in SOC was assumed to occur through uptake of carbon from the atmosphere. This is a common approach in biogeochemical modeling, see e.g. Li et al. (2006).

Characterization factors used in the present study are from the ‘CML IA baseline’ life cycle impact assessment (LCIA) method (version 3.01) as published by PRé Consultants (www.pre.nl) for use in SimaPro (the LCA software tool used in the present study). The specific method was selected because it includes the bulk of impact categories considered relevant for an agricultural LCA. As mentioned in Table 1, one category was added by the authors, namely land occupation (cf. note in Table 1). Ozone layer depletion was deselected because of little relevance in the present LCA (inclusion would only be a distraction from more relevant results). Abiotic depletion and ecotoxicity categories were deselected due to a lack of standardized impact assessment methodologies and/or data foundation. Instead, phosphorus (an abiotic resource) and toxicity as well as soil microflora were given special consideration at a semi-quantitative level.

Besides the addition of land occupation, the CML method was not updated by the authors. The CML-IA baseline method uses GWP100 values from IPCC’s 4th assessment report (AR4), i.e. 25 and 298 for methane (CH_4) and nitrous oxide (N_2O), respectively. The corresponding values in the 5th assessment report (AR5) from 2013 are, respectively, 28 and 265 without consideration of climate carbon feedback. With climate carbon feedback, the corresponding values (in the AR5) are, respectively, 28 and 298 (cf. Table 8.7 in AR5). The difference in characterization factors for N_2O (~11%) could have a minor impact on the results of the present LCA but would not change the overall conclusions.

For the chosen selection of impact categories, there is no reason to believe that the choice of other impact assessment methods would change the conclusions of the present study.

3 Inventory Analysis

Life cycle inventories for corn production with and without *P. bilaiae* in Minnesota and North Dakota were established based on four primary data sources:

1. Field trials (yield and total use of N, P, and K)
2. The ecoinvent 3 LCI database (seeds, fertilizers, pesticides, field work, and drying)
3. Results from DayCent model simulations (emissions to air and water)
4. Life cycle assessment of the *P.b.* inoculant (impacts from production)

The field trials have been described by Leggett et al. (2015)[11] and the ecoinvent database is the world's largest LCI database (ecoinvent 2014). The database contains a general process description of conventional US corn production documented in Jungbluth et al. (2007). The DayCent is described in the Methodology section and, finally, data on inoculant production came from a critically reviewed study conducted by Kløverpris et al. (2009).

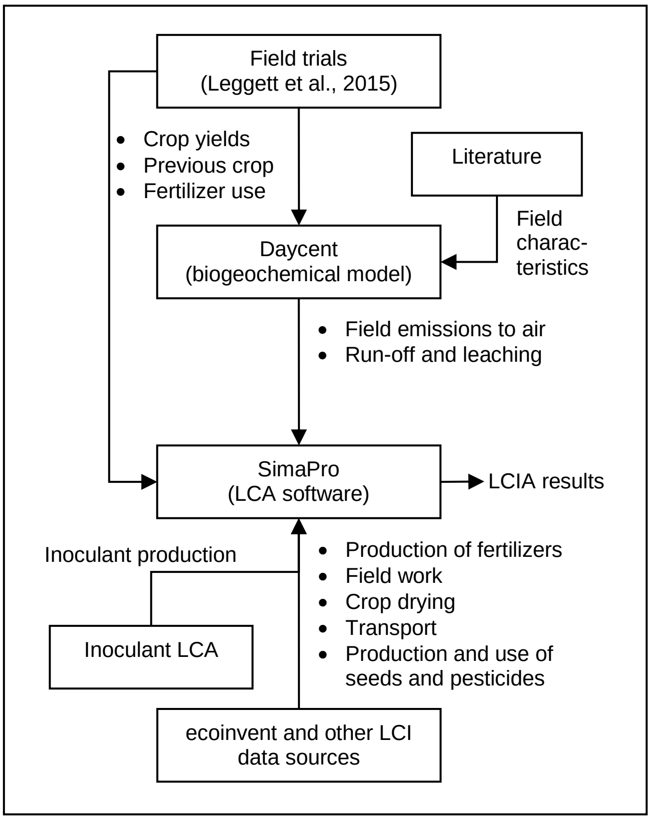

Fig. 3: Overview of data flows in the present LCA (life cycle assessment) indicating use of LCI (life cycle inventory) data and other data to obtain the LCIA (life cycle impact assessment) results

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[11] Background data from the yield increase study (Leggett et al., 2015) were also utilized in the present LCA study. These data concern unpublished information about fertilizer use. The data has been obtained through personal communication with the lead author of the yield increase study. The data has been compared to available data in the literature (described later in the present report) to ensure that it falls within expected ranges.

All inventory data were compiled in the LCA software SimaPro (version 8.0.5.13) to facilitate the conversion to resource use and environmental impacts given per metric tonne (Mg) of corn produced in the systems studied. Fig. 3 presents a graphical overview of how data were combined.

The following sections present a more detailed explanation of how data were combined to construct the life cycle inventories for the studied corn production systems.

3.1 Field trials

Leggett et al. (2015) investigated the yield effects from the application of *P. bilaiae* in US corn production for both small and large plots. In both cases, the observed yield increase was statistically significant but lower for the small plots (30-40 m^2) than for the large plots (ranging from roughly half a hectare to 32 ha). In the present study, focus is on the yield observations from the large plot field trials as this is more relevant for farming at scale, in addition to having a greater number of samples.

While Leggett et al. (2015) documented statistical differences between yields with and without the inoculant, there was no statistical difference between the yield increase obtained for corn grown after corn and corn grown after soybeans. Hence, the state-specific, average yield data applied in the present LCA was assumed to be independent of previous crop. In other words, the yield increase obtained for corn after corn (in a specific state) was assumed to the same as the yield increase for corn after soybeans. Yield data is available in Table 2.

It should be specified that the yield data in Table 2 is average yield data, which also includes data from corn fields where there was no yield response to *Penicillium bilaiae* (so-called non-responsive fields).

Background data on fertilizer rates from the large plot field trials documented by Leggett et al. (2015) were made available by Leggett (2012) to the extent available (cf. Table 2). The fertilizer data was supplemented and cross-checked with data from the literature (cf. Appendix A). The previous cropping history (corn or soybean) was also considered as this would influence the N fertilization[12] of the corn trials and thereby the results of the LCA. As for N fertilizer, field trials that had information on both previous crop and fertilizer use were utilized. However, this was only possible for Minnesota since no information on previous crop was available for North Dakota. We therefore relied on N fertilizer recommendations in the literature (further described in Appendix A). The use of P and K was considered independent of previous crop and general averages from the field trial reports containing P and K data were therefore used. Table 2 provides an overview of the applied yield and fertilizer data including the number of field trials behind each data point.

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[12] Soybeans fix nitrogen from the air and some of this remains available in the soil for next year's crop, thereby reducing the need for N fertilizer.

Table 2: Yields and fertilizer data applied in the present study including the number of field trials behind each data point

	Unit	Minnesota (MN)	N. Dakota (ND)	Number of field trials	
Yield† without *P. bilaiae*	Mg ha^{-1}	10.7	8.5	MN: 101	ND: 46
Yield† with *P. bilaiae*	Mg ha^{-1}	11.1	8.9	MN: 101	ND: 46
Total N§, corn after corn	kg ha^{-1}	181	148	MN: 12	ND: 0‡
Total N§, corn after soybean	kg ha^{-1}	158	103	MN: 13	ND: 0‡
Total P, general	kg ha^{-1}	37	19	MN: 25	ND: 20
Total K, general	kg ha^{-1}	61	19	MN: 24	ND: 9

† Yields are given in equivalents of dried corn, i.e. with a moisture content of 14%

‡ Due to lack of information on previous crop for the North Dakota field trials, the input of N fertilizer was based on Franzen (2010) as described in Appendix A.

§ Purchased mineral N fertilizer added to corn production

As presented in Table 2, the fertilizer application rates are based on a substantially lower number of field trials than the yield data. Comparisons of this data with literature (Rehm et al., 2006, Franzen, 2010) indicated that the reported values for N and P were in line with the recommendations for the region (see Appendix A).

The difference in macronutrient inputs in the two adjacent states, Minnesota and North Dakota, is explained by different yield potentials, which in turn relates to differences in climate, among other things. The different yield potentials reflect the recommended fertilizer rates in the two states.

As a note of information, Leggett et al. (2015) found that *P. bilaiae* increased yields more at large sites with low P levels than at large sites with high P levels, indicating that P fertilizer history could have an influence. In addition, Leggett et al. (2015) found that *P. bilaiae* was more effective on large plots than small plots at higher soil P levels[13]. Leggett et al. (2015) explained this by the ‘edge effect’. Along the edge of a plot, there is likely a lower density of *P. bilaiae* as compared to the center. At a small plot, the edge area will make up a larger share of the plot than in the case of a larger plot. This can explain why the larger plots generally experienced higher yield increases. While P fertilizer history may influence yield increases between different plot sizes, this did not influence the P fertilizer data in Table 2, which is based on field trial data that is largely in line with general recommendations (see Appendix A).

3.1.1 Uncertainty related to field trials

Leggett et al. (2015) report 95% confidence intervals (C.I.) for the yields in Minnesota and North Dakota as well as the deviation[14] between yields with and without the inoculant. Deviation is shown below (with 95% C.I.).

- Minnesota, deviation (yield increase with *P.b.*): 0.44 Mg ha^{-1} (0.34-0.54)
- North Dakota, deviation (yield increase with *P.b.*): 0.37 Mg ha^{-1} (0.2-0.5)

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[13] For both plot sizes, *P. bilaiae* increased yields the most at sites with low soil P levels.

[14] The deviation is the mean deviation between the yield of corn with and without the *P.b.* inoculant (all based on large plot field trials documented by Leggett et al. 2015)

The deviation expresses the statistical change in yield obtained with the *P.b.* inoculant. As it is the change that matters in consequential LCA, it is the yield deviation that has been taken into account in the modeling of the yield effect. This means that yields for the inoculant system have been calculated as the yield in the reference systems (c.f. Table 2) plus the respective yield increase with *P.b*. Note that the yield deviations are provided with two significant digits whereas subtraction of yields with and without *P.b.* in Table 2 would give a result with only one significant digit[15]. As for the modeling of the field effect in DayCent, average yields[16] with and without the inoculant were calibrated to match the yield deviations listed above (within the 40-year modeled time frame). Meanwhile, a perfect match was not obtained for corn after soybeans where the modeled average yield deviation was 0.47 Mg ha^{-1} for Minnesota and 0.39 Mg ha^{-1} for North Dakota. These minor discrepancies (5-7%) are not considered to have any appreciable impact on results and conclusions related to the corn-soybean rotations.

The uncertainty related to the yield increases (95% confidence intervals) will be considered in relation to the results of the life cycle impact assessment.

3.1.2 Yield increases in the long term

As discussed in the introduction, Penicillium bilaiae solubilizes mineral phosphorus, which allows for a better uptake of phosphorus through the crop roots and thereby better plant growth. Hence, Penicillium bilaiae solubilizes otherwise inaccessible phosphorus from the soil pool. Meanwhile, this is not expected to lead to a decrease in the mineral phosphorus pool in the long term. The reason is that the farmer needs to apply phosphorus in excess of the crop requirements due to the rapid binding of P in the mineral pool. This leads to a build-up of soil P levels (Sharpley et al. 1994). With P. bilaiae, the build-up is not avoided but the rate is slightly reduced. This means there will continue to be enough mineral P for P. bilaiae to have an effect, and the long-term yield increases are therefore not expected to be lower than those reported by Leggett et al. (2015). See also Section 4.6.1.

3.2 The ecoinvent LCI database

The life cycle inventory for average US corn production (maize grain, US, production, consequential model) in the ecoinvent database (v3.0), as described by Jungbluth et al. (2007), contains data on seeds, fertilizers, lime, pesticides, irrigation, field work, corn drying, transport, and field emissions to air and water. This information was utilized to construct LCIs for corn production in Minnesota and North Dakota as described below. Note that, since a consequential dataset was used, all sub-processes (fertilizers, etc.) were also consequential processes based on marginal data (referred to as 'market processes' in ecoinvent).

All P fertilizer (Table 2) is assumed to be applied to the field as diammonium phosphate [$(NH_4)_2HPO_4$]. This assumption is based on the applied corn dataset in ecoinvent (2014). Furthermore, it is assumed that the N not applied as diammonium phosphate is applied as a mix of liquid ammonia, urea, and ammonium nitrate as in the ecoinvent dataset (same ratio).

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[15] Example for Minnesota: 11.1 Mg ha^{-1} – 10.7 Mg ha^{-1} = 0.4 Mg ha^{-1}

[16] Yields in DayCent change from year to year because the model draws upon historic weather data where precipitation, temperature, and other factors change from year to year.

The ecoinvent dataset includes use of lime to modify soil pH. This aspect was omitted in the present study as lime contributes less than 0.25% of the environmental impacts from corn production for all categories in this study (based on the ecoinvent dataset) and hence has no significant implications for the LCA results. One could speculate that the release of organic acids from *P. bilaiae* (cf. Introduction) could result in a need for additional lime. However, the acid release does not make appreciable changes in soil acidity as it is localized right around the roots in the rhizosphere. The field trials documented by Leggett et al. (2015) were also conducted with the same amount of agricultural inputs to treated and untreated plots and, even if there were a change in the input of lime, it would likely have a very small effect on the results of the LCA (cf. the miniscule impact of lime mentioned at the start of this paragraph).

For the compilation of the inventories for corn production in Minnesota and North Dakota, it is assumed that the input of seeds as well as pesticides is the same as in the ecoinvent dataset (per hectare of cropland).

Field work in the ecoinvent dataset includes fertilizer applied by broadcasting, tillage, sowing, application of plant protection, and combine harvesting. In the present study, it is assumed that field work is the same (per hectare), acknowledging however that when *P. bilaiae* results in higher yields, slightly more energy is required for harvesting. This aspect has been ignored as it would have a negligible impact on results. Table 3 provides an overview of the inputs to corn production in the different scenarios considered.

Table 3: Overview of assumed agricultural inputs to corn production

		Corn after corn		Corn after soybeans	
Input	Unit	Minnesota	N. Dakota	Minnesota	N. Dakota
Seed	kg ha^{-1}	200	200	200	200
Ammonia, liquid	kg ha^{-1}	99	81	87	56
Urea, as N	kg ha^{-1}	42	34	36	24
Ammonium nitrate, as N	kg ha^{-1}	58	47	50	33
Phosphate fertilizer, as P_2O_5	kg ha^{-1}	85	43	85	43
Potassium chloride, as K_2O	kg ha^{-1}	73	23	73	23
Lime	kg ha^{-1}	0	0	0	0
Pesticides	kg ha^{-1}	2	2	2	2
Irrigation	m^3 ha^{-1}	0	0	0	0
Field work†	l. diesel ha^{-1}	61	61	61	61
P.b. ‡ inoculant	g ha^{-1}	6	6	6	6

† Fertilizing, tillage, sowing, application of plant protection, and harvesting
‡ *Penicillium bilaiae*

Corn drying (down to 14% moisture) and transport is also assumed to be the same as in the ecoinvent dataset, seen per Mg of corn produced.

Modeling of the production of the agricultural inputs (Table 3) and the related resource use and emissions to the environment was based on ecoinvent (2014).

3.3 DayCent simulations

The DayCent modeling has been described in the Methodology section and results will appear in the subsequent Impact Assessment chapter.

3.4 P.b. inoculant production and use

To compare the life cycle impacts of producing corn with and without *P. bilaiae*, it is necessary to know the environmental impacts of inoculant production and the dose applied. The fungus contained in the inoculant (*P. bilaiae*) is produced by 'solid state fermentation'. The fermentation medium is sterilized before inoculation with the fungus. The spores of the fungus are mixed with other ingredients. In the present study, we use the modeling of inoculant production in Kløverpris et al. (2009) but revised results (Table 4) based on updated background processes in the ecoinvent database (ecoinvent 2014). The update did not impact results substantially (compared to the original study).

Table 4: Contribution to environmental impact categories from production of one kg *P.b.* inoculant based on Kløverpris et al. (2009)

Global warming (kg CO_2 eq.)	Acidi-fication (g SO_2 eq.)	Eutro-phication (g PO_4^{3-} eq.)	Photochem. ozone form. (g C_2H_4 eq.)	Fossil energy (MJ LHV†)	Land use (m^2a)
69	360	100	20	600	12

† Mega Joule Low Heating Value

The results in Table 4 are potentially overestimated. An important aspect related to global warming is the disposal of organic waste from inoculant production. The waste is land-filled and might give rise to methane emissions. A worst-case scenario is assumed in which all the waste is degraded under anaerobic conditions and all resulting methane is released into the atmosphere. This accounts for almost 30% of the estimated contribution to global warming. Furthermore, there is some uncertainty related to the amount of natural gas (heating to maintain optimal temperature for fungal growth) and electricity used by Novozymes to produce the inoculant. Together, these two aspects account for about one-third of the climate impact from inoculant production. As the inoculant production turns out to be insignificant for the comparative LCAs of US corn production with and without *P. bilaiae*, the uncertainties discussed above are not of importance for the overall results. The dose of the inoculant amounts to roughly 5.7 g ha^{-1} (0.2 ounces applied to 80,000 seeds planted on one hectare, in line with typical seeding rates for the US Corn Belt, cf. Licht et al. 2017).

4 Impact assessment

This chapter describes the results of the life cycle impact assessment.

4.1 DayCent results

The field emissions from continuous corn to air and water as modeled using DayCent are presented in Table 5.

Table 5: Annual field emissions to air and water from continuous corn production produced with and without the use of *P. bilaiae* (*P.b.*) as modeled using DayCent (average based on a modeled 40-year time horizon, unless otherwise noted)

			Minnesota		North Dakota	
		Unit	Ref.†	*P.b.*‡	Ref.†	*P.b.*‡
Crop	Corn production§	Mg ha^{-1}	10.7	11.1	8.5	8.9
Emissions to air	Ammonia	kg NH_3 ha^{-1}	7.2	7.6	6.0	6.3
	Nitrous oxide	kg N_2O ha^{-1}	5.3	5.0	4.1	4.0
	Nitric oxide	kg $NO\cdot$ ha^{-1}	14.6	13.9	9.4	9.1
	Carbon dioxide¶	Mg CO_2 ha^{-1}	-0.7	-0.9	-0.5	-0.7
	Methane	kg CH_4 ha^{-1}	-1.9	-1.9	-2.5	-2.5
Losses to water	Phosphorus	kg P ha^{-1}	1.4	1.4	0.7	0.7
	Nitrate	kg NO_3^- ha^{-1}	173	139	52	34

† Reference system without the use of *P. bilaiae*

‡ System with use of *P. bilaiae* on corn

§ Yields are given in equivalents of dried corn, i.e. with a moisture content of 14%

¶ From change in SOC (soil organic carbon) with annual emissions based on average of first 20 years

In the systems studied, the simulations show a sequestration of SOC, as indicated by the negative CO_2 emissions from the field (Table 5). The absolute rate of C change is highly influenced by the historical practices of the system. Systems that have degraded C levels will move towards higher equilibriums when less intensive or higher producing systems are introduced and these changes can lead to C stock increases for many decades. When modeling the impact between treatments, assumptions of the cropping history are often necessary that can influence the absolute rate of C change. In this context, it is typically better to assess the relative C change between contrasting treatments rather than assessing the absolute rate of C change in each system particularly if measured C stock change is not available for site calibration. Considering this, the relative rate of increase of SOC was found to be higher with the use of *P. bilaiae* than without. The reason is that the higher yields in the inoculant systems also resulted in increased crop carbon inputs as compared to the reference systems[17]. Reduced N_2O emissions reported by DayCent were also related to improved crop production. Increased plant N uptake resulted in less surplus N remaining in the soil compared to the reference system[18]. Somewhat offsetting this, there was more

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[17] The DayCent modeling assumes that crops grow in proportion. In other words, the root-to-shoot ratio is assumed to be unchanged (in the base case analysis) and thereby the root *fraction* is also assumed to be unchanged. Meanwhile, this also implies that a higher grain yield will be accompanied by bigger crops and thereby also by bigger roots, which will result in more below-ground biomass.

[18] While increased crop production results from improved phosphorus uptake at critical growth stages, the increased crop growth also requires higher uptake of other nutrients in order to enable a uniform development of the entire plant including a constant 'root-to-shoot' ratio.

organic material and dissolved organic carbon available in the soil for denitrification (additional discussion to follow). Nevertheless, the net result was a reduction in N_2O emissions per hectare. The trace gas submodel in DayCent only considers direct losses to the atmosphere from the field. It does not compute the indirect losses that can occur from re-deposition of nitrogen from the field to surrounding areas. Overall annual rates of nitrous oxide losses were found to be in agreement with losses reported in other measurements (Wagner-Riddle et al., 1997, 2007; Johnson et al., 2010) and modeling studies (Del Grosso et al, 2006) for the region.

Lower nitrogen losses through leaching with the use of *P. bilaiae* were simulated as result of higher yields/crop-N uptake and lower levels of residual soil N. Phosphorus leaching however demonstrated no differences in losses to water between systems even though the use of *P. bilaiae* resulted in higher yields/crop-P uptake. This may have been a result of the fact that overall P losses were very small in both systems as crop demand versus nutrient availability was highly competitive. Overland nutrient runoff loses are challenging to simulate with a one-dimensional model and as such the full impact of P-losses to water may not be captured.

Methane oxidation (noted as negative emissions) was unchanged per hectare of cropland (Table 5). DayCent calculates methane oxidation as a function of soil temperature, soil water content, porosity, and field capacity and assumes the only source of CH_4 is what diffuses from the atmosphere. It is assumed that the main regulator for CH_4 oxidation rates is soil gas diffusivity as controlled by soil water content and soil physical properties (Del Grosso et al., 2000). As such, methane oxidation rates had little consequence with the agricultural practice on the field and therefore methane has been omitted from the remaining part of the study. A slight increase in NH_3 emissions (~5% seen per hectare of cropland) was estimated using the DayCent Model as a result of *P. bilaiae* use in corn production. The reason is that the ammonia emissions that occurred during harvest are strictly correlated with yields in DayCent as it assumes a fixed portion of the harvested biomass N is lost through volatilization (Del Grosso et al., 2011). The model does not characterize the conversion of urea to NH_3 from fertilizer application and thus may underestimate some of the NH_3 losses. Nitrogen emissions other than ammonia are reduced with the use of *P. bilaiae,* due primarily to increased crop yield and N uptake.

We compared the DayCent emission results in Table 5 to the same emissions in the ecoinvent dataset for US corn production. The majority of outputs were generally consistent between the two sources except in the case of predicted ammonia losses which were estimated to be ~4 kg ha^{-1} yr^{-1} lower with DayCent than with ecoinvent. The apparent strength in the modeling approach is that outputs are derived using a full mass balance for nitrogen whereas the ecoinvent dataset is aggregated together from different sources (cf. Jungbluth et al., 2007).

With the corn-soybean rotation, the impact of *P.b.* on corn was investigated for the full two-year rotation. Table 6 shows average emissions from one hectare grown with soybeans every second year and corn in the other years. Note that the emissions applies to a full crop rotation, i.e. they are the sum of emissions occurring over two years (one year with soybeans and one year with corn).

Table 6: Average crop production and emissions during one corn-soybean rotation (two years) based on DayCent (average based on a modeled 40-year time horizon, unless otherwise noted)

			Minnesota		North Dakota	
		Unit	Ref.†	*P.b.*‡	Ref.†	*P.b.*‡
Crops	Corn production§	Mg ha^{-1}	10.7	11.1	8.5	8.9
	Soybean production¶	Mg ha^{-1}	2.7	2.7	3.5	3.5
Emissions to air	Ammonia	kg NH_3 ha^{-1}	17.9	18.1	16.4	16.7
	Nitrous oxide	kg N_2O ha^{-1}	7.6	7.4	7.0	6.9
	Nitric oxide	kg NO· ha^{-1}	20.1	19.6	15.9	15.8
	Carbon dioxide#	Mg CO_2 ha^{-1}	2.3	2.0	1.3	1.1
	Methane	kg CH_4 ha^{-1}	-3.7	-3.7	-4.9	-4.9
Losses to water	Phosphorus	kg P ha^{-1}	0	0	0	0
	Nitrate	kg NO_3^- ha^{-1}	227	208	265	251

† Reference system without the use of *P. bilaiae*

‡ System with use of *P. bilaiae* on corn

§ Yields are given in equivalents of dried corn, i.e. with a moisture content of 14%

¶ Soybean yields equivalent to a 13% moisture content

From change in SOC (soil organic carbon) with annual emissions based on average of first 20 years

Methane emissions per hectare were also unaffected by the use of *P. bilaiae* for the corn-soy rotations (Table 6). Also, a small increase (1-2%) in ammonia emissions per hectare of cropland was observed while all other nitrogen emissions decreased. The DayCent model was able to reasonably simulate a mass balance of nitrogen flows (details available in Appendix B).

As previously discussed, CO_2 emissions from changes in SOC are dependent on the time perspective because SOC levels are known to move from one equilibrium stage towards another when management practices are changed, e.g. when *P. bilaiae* is introduced. The use of *P. bilaiae* resulted in a higher level of SOC seen over a 20-year time horizon when compared to the reference system (Table 5 and Table 6). This is due to increased crop production with a higher crop carbon input resulting in increased carbon sequestration in the soil. While CO_2 emissions from SOC are generally lower with the use of *P. bilaiae*, the difference between the reference systems and the corresponding *P. bilaiae* systems generally become smaller with time (Table 7). This should also be expected due to the progression towards equilibrium discussed above and in Section 2.2.6.3.

Table 7: Average soil CO_2 emissions (Mg CO_2 ha^{-1}) from change in SOC (soil organic carbon) through one crop cycle (one year for continuous corn and two years for corn-soy rotations)

	Minnesota				North Dakota			
Time	Continuous corn		Corn-soybeans		Continuous corn		Corn-soybeans	
horizon	Ref.†	*P.b.*‡	Ref.†	*P.b.*‡	Ref.†	*P.b.*‡	Ref.†	*P.b.*‡
10 years	-1.45	-1.67	2.10	1.79	-1.51	-1.72	-0.43	-0.60
20 years	-0.69	-0.86	2.27	2.04	-0.49	-0.68	1.30	1.15
30 years	-0.54	-0.69	1.60	1.42	-0.46	-0.62	0.91	0.77
40 years	-0.33	-0.46	1.58	1.43	-0.20	-0.34	0.98	0.85

† Reference system without the use of *P. bilaiae*

‡ System with use of *P. bilaiae* on corn

In agricultural LCA, N_2O field emissions are usually assumed to be linearly correlated with N inputs. However, there is some interplay between N_2O emissions and the (time-dependent) SOC level (cf. discussion of DayCent results following Table 5). This means that the application of a 20-year time perspective for SOC (applied for the reasons discussed in Section 2.2.6.3) is not fully consistent with the 40-year averages applied for other emissions, including N_2O. Hence, average N_2O emissions for the first 20 years of DayCent simulations were also calculated (data not shown) to derive N_2O results consistent with the 20-year SOC results. While this did impact N_2O results, the direction of the impact was not consistent. The reduction in N_2O emissions obtained with the inoculant increased in three of the four investigated case studies whereas it decreased in the last (corn after soybeans in North Dakota). In this context, it is important to keep in mind that N_2O emissions can be highly influenced by single events (e.g. heavy rainfall) and that a longer time perspective will seek to level out impacts of such events and thereby generate a more generic picture. Meanwhile, differences in average N_2O emissions over a 20- and 40-year time perspective also illustrates the uncertainties related to N_2O modeling. This aspect will later be explored in the uncertainty analysis.

4.2 Life cycle GHG assessment for continuous corn

The life cycle impacts from continuous corn production in Minnesota and North Dakota were estimated for the reference system as well as the inoculant system (cf. Fig. 1). Results for the global warming impact category (GHG emissions) are shown in Table 8 (Minnesota) and Table 9 (North Dakota).

Table 8: Life cycle greenhouse gas emissions for continuous corn (14% moisture) in Minnesota (kg CO_2e per Mg corn)

		Reference	Inoculant system			Difference	
Categories	Reference Flows	corn (1 Mg)	*P.b.* corn‡ (1.041 Mg)	Displaced corn§ (-0.041 Mg)	Total (1 Mg)	(Total *P.b.* vs. Ref.¶) Abs.	Rel.
Field emissions†	CO_2 (20 y avg.)	-64.1	-80.8	-	-80.8	-16.7	N/A#
	N_2O	147.3	140.3	-6.06	134.2	-13.1	-8.9%
Inputs to the field	Corn seeds	31.0	31.0	-1.28	29.8	-1.3	-4.1%
	N fertilizers	80.7	80.7	-3.32	77.4	-3.3	-4.1%
	P fertilizer	14.9	14.9	-0.61	14.3	-0.6	-4.1%
	K fertilizer	3.8	3.8	-0.15	3.6	-0.2	-4.1%
	Pesticides	2.3	2.3	-0.09	2.2	-0.1	-4.1%
	Field work	28.6	28.6	-1.18	27.4	-1.2	-4.1%
	Inoculant		0.04	-	0.04	0.04	N/A
Post treatment	Drying	12.455	12.967	-0.51	12.5	0.0	0.0%
	Transport	1.171	1.219	-0.05	1.2	0.0	0.0%
Total		258.0	234.9	-13.24	221.7	-36.4	-14.1%

† DAYCENT results
‡ Corn grown with *Penicillium bilaiae*
§ Displaced corn grown without *Penicillium bilaiae* (CO_2 field emissions set to zero, cf. discussion in text)
¶ Reference system without the use of *Penicillium bilaiae*
# Since the CO_2 emissions from SOC in the reference system are also negative, the relative change (+26%) does not provide a useful number.

Table 9: Life cycle greenhouse gas emissions for continuous corn (14% moisture) in North Dakota (kg CO_2e per Mg corn)

		Reference corn (1 Mg)	Inoculant system			Difference (Total *P.b.* *vs.* Ref.¶)	
Categories	Reference Flows		*P.b.* corn‡ (1.044 Mg)	Displaced corn§ (-0.044 Mg)	Total (1 Mg)	Abs.	Rel.
Field emissions†	CO_2 (20 y avg.)	-57.7	-79.5	-	-79.5	-21.8	N/A#
	N_2O	142.9	138.8	-6.22	132.6	-10.3	-7.2%
Inputs to the field	Corn seeds	39.1	39.1	-1.70	37.4	-1.7	-4.4%
	N fertilizers	83.0	83.0	-3.61	79.4	-3.6	-4.4%
	P fertilizer	9.3	9.3	-0.41	8.9	-0.4	-4.4%
	K fertilizer	1.5	1.5	-0.06	1.4	-0.1	-4.4%
	Pesticides	2.9	2.9	-0.12	2.7	-0.1	-4.4%
	Field work	36.0	36.0	-1.57	34.4	-1.6	-4.4%
	Inoculant		0.05	-	0.05	0.05	N/A
Post treatment	Drying	12.5	13.0	-0.54	12.5	0.0	0.0%
	Transport	1.2	1.2	-0.05	1.2	0.0	0.0%
Total		270.6	245.3	-14.29	231.0	-39.5	-14.6%

† DAYCENT results
‡ Corn grown with *Penicillium bilaiae*
§ Displaced corn grown without *Penicillium bilaiae* (CO_2 field emissions set to zero, cf. discussion in text)
¶ Reference system without the use of *Penicillium bilaiae*
# Since the CO_2 emissions from SOC in the reference system are negative, the relative change (+38%) does not provide a useful number.

The GHG emissions for the reference system in Table 8 and 9 were estimated by use of standard LCA procedure, i.e. all impacts from inputs (e.g. grain and fertilizer), field emissions (e.g. N_2O), and impacts from transport and drying of the fresh corn were summed and then related to the functional unit (1 Mg of dried corn kernels). Note that CO_2 field emissions are negative. This is explained by an ongoing build-up of organic carbon in the soil (cf. Table 5).

The GHG results for the inoculant system in Table 8 and 9 have been specified by separating out the (negative) emissions from displaced corn (of which the categories ‘Field emissions’ and ‘Inputs to the field’ make up the yield effect). Note that for each Mg of corn produced in the reference system, the same area of cropland will produce a higher amount of corn in the inoculant system. The additional yield will then displace a corresponding amount of corn elsewhere so the total production will equal that in the reference system. As mentioned, in the methodology section, the base case assumption is that displaced corn will have the same characteristics as corn produced in the reference system. Hence, the same dataset as for the reference system is used when estimating the emissions from displaced corn (and thereby the yield effect). There is, however, one exception: The change in soil organic carbon (CO_2 field emissions) is set to zero in the displaced corn production system. The reason is as follows. The displacement of corn production elsewhere frees up agricultural land for other purposes. In an all-else-equal scenario, the freed-up land in the relevant region will leave productive use and start to undergo natural succession, which would lead to carbon sequestration in the soil (exceeding the carbon sequestration shown in the reference system in Table 5). Meanwhile, freed up agricultural land is probably more likely to stay in production due to the continued increase in the global demand for food. In this view and seen in the context of a still increasing global agricultural area, the land saving obtained per Mg of corn produced with *P. bilaiae* may not free up land but instead delay ongoing land conversion from natural vegetation to agriculture (Kløverpris and Mueller 2013). In this perspective, the ‘freed up’ land is likely to reflect prevented or delayed land conversion somewhere else and thereby prevented or delayed GHG emissions from decomposition of natural vegetation. In any case (whether land is freed up or land transformation is prevented or delayed), there is a reduced impact in terms of global warming. This aspect has however been ignored in the base case analysis by the assumption of no change in SOC in the displaced corn production (cf. Table 8 and 9). Thereby, it is only the impacts from avoided inputs (and their associated emissions) that are considered in the base case yield effect – not the land saving effect in terms of avoided GHG emissions from land use change. This can be considered conservative in the sense that it does not favor the *P. bilaiae* inoculant.

The two right-hand columns in Table 8 and 9 show the absolute and the relative difference in GHG emissions between the reference system and the inoculant system for continuous corn in Minnesota and North Dakota, respectively. Note that the relative difference is constant when it comes to ‘inputs to the field’ (the inoculant being the exception because it is only added in the *P. bilaiae* systems). This is to be expected because the inputs are directly proportional to the area cultivated. With a higher yield, less land is required to produce 1 Mg of corn and the inputs are thereby reduced proportionally. Note also that there is no difference in drying and transport GHG emissions between the inoculant systems and their respective reference systems. This is also to be expected when looking at 1 Mg of dried corn as the functional unit.

The differences between Minnesota and North Dakota appearing from Table 8 and 9 are mainly explained by different climatic conditions (affecting the field emissions modeled with DayCent), different initial corn yields (before application of *P. bilaiae*), and different relative yield increases (from the use of *P. bilaiae*). The *P.b.* inoculant is applied as a constant dose per hectare (cf. Table 3) and the related impacts per Mg of corn produced in the system are therefore affected by the initial corn yield. Impacts related to displaced corn production elsewhere[19] are governed by the relative yield increase (determining how much corn is displaced elsewhere per Mg of corn produced at the inoculated field). This explains, at the overall level, the differences in results between Table 8 and 9.

The rather complex overview in Table 8 and 9 can be aggregated into the three main categories previously discussed in the Methodology section, i.e. upstream effects, the field effect, and the yield effect (aggregate results to appear later in the report). Transport and drying are not included in the main categories above because these processes have no influence on the difference between the reference system and the inoculant system (cf. Table 8 and 9). It is however important to include these processes when considering the relative change of the impact of corn production when *P. bilaiae* is introduced. As indicated in Table 8 and 9, the GHG impact of 1 Mg dried continuous corn is reduced by 14-15% when *P. bilaiae* is introduced in Minnesota and North Dakota, respectively. The changes in field emissions (CO_2 and N_2O) make up the largest contributors to these reductions, which shows how important nutrient efficiency and plant growth is for the sustainability of crop production.

4.3 Life cycle GHG assessment for corn after soybean

The environmental consequences of using *P. bilaiae* on corn after soybean (in a corn/soybean rotation) were estimated based on the system considerations laid out in the Methodology section. Hence, the reference system consists of corn being produced in a corn-soybean rotation without *P. bilaiae*. The inoculant system consists of corn produced with *P. bilaiae* in a corn soybean rotation on the same area as in the reference system. The additional corn yield in the inoculant system is assumed to replace corn production elsewhere (cf. Fig. 2). To handle continuous corn and corn-soybean rotations consistently, it is assumed that additional corn production in the inoculant system displaces continuous corn produced without *P. bilaiae*, i.e. corn with the same characteristics as the continuous corn reference system. By this approach, the environmental benefits from a yield increase of 1 kg corn (the yield effect) is the same regardless of which crop rotation the additional corn comes from (as should logically be expected).

As also mentioned in the Methodology section, the full crop rotation (corn and soybeans) is included in the system to account for the full field effect, i.e. the changes in emissions from the field occurring as a result of introducing *P. bilaiae* on corn. The inputs to soybean production as well as the output (the soybeans) cancel out when the reference system is ‘subtracted’ from the inoculant system. This is because the yield of soybeans is assumed not to be affected of whether the inoculant is used on the corn crop grown on the field the previous year or not.

While the procedure described above allows for the modeling of changes in environmental impacts per Mg of corn when *P. bilaiae* is introduced, it does not provide an estimate of

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[19] The yield effect

the environmental impacts of corn production in the reference (corn-soybean) system. Due to the integrated nature of a corn-soybean rotation, it is challenging to determine the environmental impacts of producing the corn alone. For instance, the soybean fixes nitrogen in the soil, which is then used by the corn in the next year. One could look at inputs to the system and emissions from it during years with corn production alone but this would result in a somewhat artificial allocation of environmental impacts among the corn and soybean due to the close integration of the two crops. In addition, the only purpose of this exercise would be to estimate the 'reference corn impacts' to allow for an assessment of the relative change in impacts when *P. bilaiae* is introduced. Instead of embarking on this attempt, the reference impacts from continuous corn production were simply used as the benchmark to which the change in impacts caused by *P. bilaiae* on corn after soybeans was compared. Table 10 shows the estimated changes in GHG emissions (in the form of the main categories previously discussed) when *P. bilaiae* is introduced in corn after soybeans. Table 10 also summarizes the results for continuous corn (previously shown in Table 8 and 9).

Table 10: Change in life cycle greenhouse gas (GHG) emissions with the use of *P. bilaiae* (*P.b.*) on corn (kg CO_2e per Mg corn) - base case results

	Continuous corn		Corn after soybean	
	Minnesota	N. Dakota	Minnesota	N. Dakota
Upstream effect (*P.b.* production)	0.04	0.05	0.04	0.05
Field effect, CO_2†, 20-year avg.	-16.7	-21.8	-21.9	-18.3
Field effect, N_2O	-7.0	-4.1	-4.6	-1.9
Yield effect (displacement)‡§	-12.7	-13.7	-12.7	-13.7
Total change with *P. bilaiae* on corn	-36.4	-39.5	-39.2	-33.9
Continuous corn without *P.b.* (benchmark)	258.0	270.6	258.0	270.6
Reduction with *P.b.* versus benchmark	14.1%	14.6%	15.2%	12.5%

† From change in SOC (soil organic carbon)
‡ Displaced crop production elsewhere including inputs here fore (avoided land use emissions from reduced land occupation omitted in the base results)
§ The yield effect does not include changes in post treatment (drying and transport)

The field effects (CO_2 and N_2O) for corn after soybean (Table 10) are based on the information in Table 6. As expected, the yield effect was the same for both continuous corn and corn after soybean within each state (Table 10) since the (absolute) yield increases were identical. In addition, the production of the *P.b.* inoculant only acted as a minor contributor towards the overall GHG results. Uncertainty of the results has been addressed in Section 5.1.1.

4.4 *Life cycle impact assessment for other impact categories*

All impact categories considered in the present study are presented in Table 11.

Table 11: Change in impacts per Mg corn produced with *P. bilaiae* (base case results)

System	Impact category	Unit	Up-stream¶	Field effect	Yield effect§	Total change	Relative change†
Corn after corn, Minne-sota	Fossil energy resources	MJ LHV‡	0.32	0	-81	-81	-3.8%
	Global warming	kg CO_2e	0.04	-24	-13	-36	-14.1%
	Photochem. oxidation	g C_2H_4e	0.01	0	-2	-2	-2.9%
	Acidification	g SO_2e	0.19	27	-117	-90	-2.9%
	Eutrophication	g PO_4^{3-}e	0.06	-318	-142	-460	-12.8%
	Land occupation	m^2a	0.01	0	-25	-25	-3.1%
Corn after corn, North Dakota	Fossil energy resources	MJ LHV‡	0.40	0	-91	-90	-4.0%
	Global warming	kg CO_2e	0.05	-26	-14	-40	-14.6%
	Photochem. oxidation	g C_2H_4e	0.01	0	-2	-2	-3.0%
	Acidification	g SO_2e	0.24	38	-122	-84	-2.8%
	Eutrophication	g PO_4^{3-}e	0.07	-209	-103	-312	-12.5%
	Land occupation	m^2a	0.01	0	-33	-33	-3.4%
Corn after soy, Minne-sota	Fossil energy resources	MJ LHV‡	0.32	0	-81	-81	-3.8%
	Global warming	kg CO_2e	0.04	-27	-13	-39	-15.2%
	Photochem. oxidation	g C_2H_4e	0.01	0	-2	-2	-2.9%
	Acidification	g SO_2e	0.19	13	-117	-104	-3.4%
	Eutrophication	g PO_4^{3-}e	0.06	-180	-142	-322	-9.0%
	Land occupation	m^2a	0.01	0	-25	-25	-3.1%
Corn after soy, North Dakota	Fossil energy resources	MJ LHV‡	0.40	0	-91	-90	-4.0%
	Global warming	kg CO_2e	0.05	-20	-14	-34	-12.5%
	Photochem. oxidation	g C_2H_4e	0.01	0	-2	-2	-3.0%
	Acidification	g SO_2e	0.24	38	-122	-84	-2.8%
	Eutrophication	g PO_4^{3-}e	0.7	-158	-103	-261	-10.4%
	Land occupation	m^2a	0.1	0	-33	-33	-3.4%

† Total compared to continuous corn without *P.b.* (within the same state) incl. transport and drying
‡ Mega Joule Low Heating Value
§ Displacement approach
¶ Production of *P.b.* inoculant

For every impact category considered, a reduction was observed when introducing *P. bilaiae* in US corn production (no trade-offs between impact categories). The greatest reductions were seen for the global warming and eutrophication impact categories (respectively 12-15% and 9-13% reduction compared to continuous corn production without the use of *P. bilaiae*). There was a substantial field effect from *P. bilaiae* for these two impact categories (cf. reduction in nitrate losses to the aquatic environment in Table 5 and 6). For the other impact categories, the reductions in environmental impacts are more modest (2.8-4.0%) and almost entirely related to the yield effect (with a small upstream impact from the *P.b.* inoculant).

The thorough reader will notice that there is a small positive contribution from the field effect to the acidification impact category. This is due to the increase in ammonia emissions from the field where *P. bilaiae* is applied (cf. Table 5 and 6). As mentioned in

Section 4.1, ammonia emissions are strictly tied to yield in DayCent. Hence, when yields go up, so do ammonia emissions. Meanwhile, this increase is mirrored by a corresponding reduction in ammonia emissions related to the displacement of corn elsewhere. On top of this, the displaced corn production (including the displaced agricultural inputs) also results in many other avoided contributions to acidification, which means that the net effect is an overall reduction in acidifying emissions as outlined in Table 11 (‘Total change’).

4.5 *Simplified formula for global warming results*

The previous results rely on a detailed analysis, which may not always be feasible when considering environmental impacts of the *P.b.* inoculant. To broaden the applicability of the present study, a simplified formula has been derived from the base case results to give a rough estimate of global warming results based on a few input parameters. The purpose of this has been to allow for a quick assessment of GHG impacts related to future field trials conducted by farmers, research communities, and/or other stakeholders. A similar formula could be derived for the other impact categories. The global warming formula looks as follows.

- $\Delta GWP_{100} = \Delta GWP_{100,\text{ upstream}} + \Delta GWP_{100,\text{ field}} + \Delta GWP_{100,\text{ yield}}$ (Equation 1)

  where ΔGWP_{100} is the change in global warming impact per Mg corn

The remaining terms in Equation 1 have been defined below. Equation 2 describes the upstream effect.

- $\Delta GWP_{100,\text{ upstream}} = 0.39 \text{ kg } CO_2e \text{ ha}^{-1} / Y_{c,i}$ (Equation 2)

  where $Y_{c,i}$ is the initial corn yield (before use of the *P.b.* inoculant) per hectare

The 0.39 kg CO_2e ha^{-1} in Equation 2 is the dose of inoculant per hectare (Table 3) multiplied by the life cycle GHG emissions from production of the inoculant (Table 4).

Equation 3 describes the field effect.

- $\Delta GWP_{100,\text{ field}} = \Delta Y_c / (0.415 \text{ Mg ha}^{-1}) \cdot (-24.1 \text{ kg } CO_2e \text{ Mg}^{-1})$ (Equation 3)

  where ΔY_c is the yield increase (obtained with the *P.b.* inoculant) per hectare

The 0.415 Mg ha^{-1} in Equation 3 is the average yield increase from *P. bilaiae* applied in the DayCent modeling to estimate the yield effect (see Section 3.1.1). The -24.1 kg CO_2e Mg^{-1} is the average field effect for global warming (CO_2 and N_2O) in the base case[20]. Note that Equation 3 assumes a linear relation between yield increase and field effect. While this is an approximation, which only applies to realistically obtainable yield increases, the assumption is supported by previous biogeochemical modeling (Kløverpris et al. 2009). In the previous study, a field effect of -21 kg CO_2e per Mg corn was modeled for a five

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[20] The number is calculated by summing up the field effect results in Table 10 for CO_2 (SOC) and N_2O for all four cases (i.e. eight figures in total) and then dividing by four: (-16.7 + (-21.8) + (-21.9) + (-18.3) + (-7.0) + (-4.1) + (-4.6) + (-1.9)) kg CO_2e Mg^{-1} / 4 = -24.1 kg CO_2e Mg^{-1}

percent yield increase[21]. When raising the yield increase from five to six percent, a field effect of -25 kg CO_2e per Mg corn was estimated[22] (using the same modeling approach). Hence, a 20% increase in yield increase resulted in approximately the same relative increase in field effect thereby supporting the assumption of proportionality outlined in Equation 3.

Equation 4 describes the yield effect.

- $\Delta GWP_{100,\text{ yield}} = -\Delta Y_c / Y_{c,i} \cdot GWP_{100,\text{ ref.}}$ (Equation 4)

  where $GWP_{100,\text{ ref.}}$ is the GWP_{100} of one Mg reference corn (cultivation only[23])

The yield effect is expressed on the basis of initial yield, the yield increase, and the impact of corn cultivation in the reference system. The average GHG impact for corn cultivation in the reference systems in the present study (Minnesota and North Dakota) is 312 kg CO_2e Mg^{-1} – based on N_2O emissions and 'Inputs to the field' in Table 8 and 9 (CO_2 field emissions omitted for the reasons discussed in Section 4.2)[24]. For comparison, the corresponding number for US corn in the ecoinvent database is 374 kg CO_2e Mg^{-1} ('Maize grain {US}| production | Conseq, U', impacts from drying and transport subtracted[25]).

In addition to the equations above, a formula for estimating the combined upstream effect and field effect per hectare for global warming has also been derived. This is based on the average of the field emissions listed in Table 5 and 6.

Equation 5 describes the combined upstream and field effects per hectare.

- $\Delta GWP_{100,\text{ upstream and field, area-based}} =$

$$0.39 \text{ kg } CO_2e \text{ ha}^{-1} - \Delta Y_c / (0.415 \text{ Mg ha}^{-1}) \cdot 232 \text{ kg } CO_2e \text{ ha}^{-1} \quad \text{(Equation 5)}$$

The 0.39 kg CO_2e ha^{-1} is the upstream impact per hectare (cf. Equation 2) and the 232 kg CO_2e ha^{-1} is the average (numerical) difference in field emissions (N_2O and CO_2) between inoculated and non-inoculated corn production listed in Table 5 and 6 (converted to CO_2 equivalents)[26].

Equation 5 can be used when estimating the impact of using *P.b.* on a specific area of cropland without considering potential displacement of crop production elsewhere. If the

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[21] See last paragraph in Section 7.1.1 in Kløverpris et al. (2009)

[22] See Table 13 (0% increased root fraction) in Kløverpris et al. (2009)

[23] Transport, drying, and CO_2 from changes in SOC not included

[24] In other words, the average GHG impact for corn cultivation in the reference system is calculated by subtracting CO_2 field emissions (in the reference system) from total GHG emissions in the reference system. A calculation example for continuous corn in Minnesota (based on Table 8) is shown here: 258 kg CO_2e Mg^{-1} – (-64 kg CO_2e Mg^{-1}) = 322 kg CO_2e Mg^{-1}

[25] The ecoinvent dataset does not involve CO_2 emissions from changes in SOC

[26] Calculation example for continuous corn in Minnesota (based on Table 5): (5.0 – 5.3) kg N_2O ha^{-1} · 298 kg kg CO_2e / kg N_2O + (-0,9 – (-0.7)) Mg CO_2e ha^{-1} · 10^3 kg/Mg = -254 kg CO_2e ha^{-1}. While the sign of the result is negative, it is the numeric average of the four cases that has been used in Equation 5. This is because the negative sign (in Equation 5) has been placed in front of the second term of the formula (to simplify it).

area is A, then the combined upstream and field effects can be estimated by multiplying A with $\Delta GWP_{100, \text{upstream and field, area-based}}$.

In US units, Equation 5 will look as follows:

- $\Delta GWP_{100, \text{upstream and field, area-based}}$ =

  $$0.16 \text{ kg } CO_2e \text{ acre}^{-1} - \Delta Y_c / (6.61 \text{ bushels acre}^{-1}) \cdot 94 \text{ kg } CO_2e \text{ acre}^{-1}$$

  where ΔY_c is the yield increase expressed in bushels per acre.

4.6 Impacts not covered by the quantitative LCIA

This present section seeks to add perspectives on potential impacts not covered by the quantitative life cycle impact assessment.

4.6.1 Phosphorus

Phosphorus is a finite resource. It is also of vital importance to modern crop production. The *P.b.* inoculant saves phosphorus resources by enabling more crop production from the same amount of P fertilizer. This is possible due to more effective use of the P fertilizer applied. According to Cordell et al. (2009), roughly one-third of the phosphorus applied to arable land is lost through erosion to inland and/or coastal waters. Due to the rapid mineral binding of phosphorus in the soil, the P losses to the aquatic environment primarily occur in the form of mineral phosphate. By solubilizing some of the mineral phosphate close to the crop roots, *P. bilaiae* allows for a higher P uptake and a reduced loss of P to the environment. That is why there is no additional need for P fertilizer where the inoculant is applied.

Whether there is an impact on the soil P pool of any significance in the long term is unclear. However, the soil P pool is likely to be governed by much stronger mechanisms than an increased level of *P. bilaiae* in the root zone. These mechanisms include erosion (as mentioned) and the degree of phosphorus saturation in the specific agricultural soil.

The phosphorus savings obtained with *P. bilaiae* (per Mg corn) could potentially be off-set by use of phosphorus resources in the upstream production of the inoculant. Meanwhile, this aspect was investigated by Kløverpris et al. (2009) and the P use in the cradle-to-gate production of the inoculant turned out to be orders of magnitude lower than the P savings obtained through the increase in crop yield. Hence, it is safe to conclude that the *P.b.* inoculant saves P resources.

The commercial *P.b.* products on the market do not come with a recommendation to decrease use of P fertilizer. Meanwhile, farmers will monitor the effectiveness of their fertilizer based on their observations and adjust as they see fit.

4.6.2 Soil microflora

Trabelsi and Mhadi (2013) did a review on microbial inoculants and their impact on soil microbial communities. They found that microbial inoculants can impact the soil microflora temporarily and in the long term. They also state that these effects are still not well understood and need further research. Meanwhile, the review by Trabelsi and Mhadi (2013) did not cover *P. bilaiae*. In addition, it is important to keep in mind that there are several inoculation techniques for microbes applied in crop production, e.g. inoculation in the planting furrow and spray inoculation on the soil (Fukami et al. 2016). *P. bilaiae* is applied as a seed treatment, i.e. a quite targeted form of inoculation. Gómez-Muñoz et al. (2017) studied effects of *P.b.* on corn in a rhizobox[27] experiment. They found that the *P.b.* inoculant led to higher root volumes and that the microbe could not be detected in the rhizosphere after 27 days of growth, only close to the seed (the place of inoculation). This could indicate that the impact of the *P.b.* inoculant occurs early in the crop life cycle after which the microbe is depressed by other soil organisms. However, more research is required to support this theory. In any case, the literature does not seem to mention any adverse impacts on soil microflora specifically from the use of *P. bilaiae*. Interestingly, Sharma et al. (2013) make the point that P fertilizers cause adverse environmental impacts on overall soil health. Keeping in mind that the *P.b.* inoculant effectively reduces the amount of P fertilizer per Mg corn produced, it may actually have a positive net effect on soil health (in a full life cycle perspective). Meanwhile, more research is required to fully understand the impact of *P.b.* on microflora.

4.6.3 Toxicity

The use of the *P.b.* inoculant effectively reduces the use of pesticides per Mg of corn produced. Seen in isolation, this represents a reduction in toxicity. The question is then whether this is off-set by the upstream effect (the production of the inoculant) and/or the use of the inoculant. This is highly unlikely. The inoculant is produced in a contained environment through a biological process (solid state fermentation) without the use of hazardous chemicals. The inoculant is not toxic. On the contrary, it is designed to promote plant growth through the viability and development of the fungal spores contained in it.

4.6.4 Water

The development of bigger crops with higher yields will likely lead to higher evapotranspiration from the specific field and thereby a higher ‘green’ water footprint. In case the crops are fully rain-fed, this is not a major issue. If the crops are irrigated, consumptive water use (the ‘blue’ water footprint) may increase. Meanwhile, it is important to keep in mind that the higher yields in one place while replace crop production in another place (all-else-being-equal). Hence the net water use is likely to be more or less unaffected per Mg of corn produced. Some water is used in production of the inoculant (upstream effect) but this is likely off-set by the water use saved (through the yield effect) from reduced use of agricultural inputs per Mg corn produced.

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[27] A box designed for plant growth experiments

5 Data quality assessment

A qualitative assessment of the quality and importance of the reference flows of the present LCA study.

The data quality has been assessed with a view to the 10 data quality areas listed in ISO (2006b), namely time related coverage, geographical coverage, technology coverage, precision, completeness, representativeness, consistency, reproducibility, sources of the data, and uncertainty of the information.

Four data quality scores have been considered, namely ‘very good’, ‘good’, ‘fair’, and ‘poor’. The scores have been based on an overall assessment of the 10 areas listed above.

The importance of each reference flow has been based on the observed impact on final results. Here, the following three options have been considered: Low, medium, and high.

The outcome of the qualitative assessment is available in Table 12.

Table 12: Qualitative assessment of data quality and importance

Reference flows	Data sources	Data Quality	Importance
Seeds	ecoinvent (2014)	Good	Medium
N fertilizers†	ecoinvent (2014)	Good	Medium
P fertilizer	ecoinvent (2014)	Good	Low
K fertilizer	ecoinvent (2014)	Good	Low
Pesticides	ecoinvent (2014)	Good	Low
Field work	ecoinvent (2014)	Good	Medium
Drying of fresh corn	ecoinvent (2014)	Good	Low
Transport of corn	ecoinvent (2014)	Good	Low
Inoculant production	Kløverpris et al. (2009)	Good	Low
Carbon flows (field)	DayCent modeling	Good	High
Nitrogen flows (field)	DayCent modeling	Good	High

† Liquid ammonia, urea, and ammonium nitrate

As shown in Table 12, the data quality of all reference flows is considered good. The characterization of importance is a generic assessment. For instance, the carbon and nitrogen flows modeled with DayCent are of high importance for global warming and eutrophication but has no impact on fossil energy resources, photochemical oxidation, and land use (cf. Table 11).

Due to the high importance of the carbon flows (impacting CO_2 emissions related to changes in soil organic carbon) this topic will receive a great deal of attention in the sensitivity analyses, which will also explore an alternative approach to modeling of the yield effect.

5.1 Uncertainty analysis of global warming results (base case)

To identify and address key elements of uncertainty related to the global warming results, the simplified formula (cf. Section 4.5) was slightly expanded as explained below.

The formula for the upstream effect (Equation 2) was unchanged whereas the formula for the field effect (Equation 3) and the yield effect (Equation 4) were both split into two sub terms (see below).

The formula for the field effect (Equation 3) was expanded to include separate terms for the ‘CO_2 field effect’ ($\Delta GWP_{100,\text{ field, SOC}}$) and the ‘$N_2O$ field effect’ ($\Delta GWP_{100,\text{ field, N2O}}$).

- $\Delta GWP_{100,\text{ field}} = \Delta GWP_{100,\text{ field, SOC}} + \Delta GWP_{100,\text{ field, N2O}}$ (Equation 6)

Equation 7 describes the ‘CO_2 field effect’.

- $\Delta GWP_{100,\text{ field, SOC}} = \Delta Y_c \cdot GWP_{100,\text{ SOC}}$ (Equation 7)

  where $GWP_{100,\text{ SOC}}$ is the specific CO_2 emissions per Mg corn [kg CO_2e Mg^{-1}] from changes in SOC given per Mg change in yield per hectare [Mg ha^{-1}]

To elaborate slightly on $GWP_{100,SOC}$, it expresses how much CO_2 is released from the organic carbon pool when the corn yield increases by one Mg per hectare[28]. The unit of $GWP_{100,\text{ SOC}}$ thereby becomes kg CO_2e Mg^{-1} / Mg ha^{-1} = kg CO_2e ha Mg^{-2}.

Equation 8 describes the ‘N_2O field effect’.

- $\Delta GWP_{100,\text{ field, N2O}} = \Delta Y_c \cdot GWP_{100,\text{ field, N2O}}$ (Equation 8)

  where $GWP_{100,\text{ field, N2O}}$ is the specific GHG emissions per Mg corn [kg CO_2e Mg^{-1}] from changes in N_2O emissions given per Mg change in yield per hectare [Mg ha^{-1}]

In other words, $GWP_{100,\text{ field, N2O}}$ expresses how much N_2O emissions are reduced (in CO_2 equivalents) when the corn yield increases by one Mg per hectare. As for $GWP_{100,\text{ SOC}}$, the unit of $GWP_{100,\text{ field, N2O}}$ is kg CO_2e ha Mg^{-2}.

The formula for the yield effect (Equation 4) was also expanded to include two separate terms where one describes the emissions from displaced agricultural inputs to the field and the other describes the avoided N_2O (field) emissions from displaced use of N fertilizer (cf. Table 8 and 9).

- $\Delta GWP_{100,\text{ yield}} = \Delta GWP_{100,\text{ yield, inputs}} + \Delta GWP_{100,\text{ yield, N2O}}$ (Equation 9)

Equation 10 describes the yield effect related to displaced agricultural inputs.

- $\Delta GWP_{100,\text{ yield, inputs}} = \Delta Y_c \cdot GWP_{100,\text{ inputs}}$ (Equation 10)

  where $GWP_{100,\text{ inputs}}$ is the specific GHG emissions per Mg corn [kg CO_2e Mg^{-1}] from inputs displaced elsewhere in the expanded system (cf. Fig. 1 and 2) given per Mg change in yield per hectare [Mg ha^{-1}][29]

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[28] Hence, if $GWP_{100,SOC}$ is negative, it indicates sequestration of carbon in the soil pool.

[29] Note that, since $GWP_{100,\text{ inputs}}$ expresses emissions from displaced inputs, the value is negative.

Equation 11 describes the yield effect related to reduced N_2O (field) emissions from displaced use of N fertilizer.

- $\Delta GWP_{100,\ yield,\ N2O} = \Delta Y_c \cdot GWP_{100,\ yield,\ N2O}$ (Equation 11)

  where $GWP_{100,\ yield,\ N2O}$ is the specific GHG emissions per Mg corn [kg CO_2e Mg^{-1}] from avoided N_2O emissions in the displaced corn system given per Mg change in yield per hectare [Mg ha^{-1}][30]

A graphical overview of the symbols discussed above has been presented in Fig. 4.

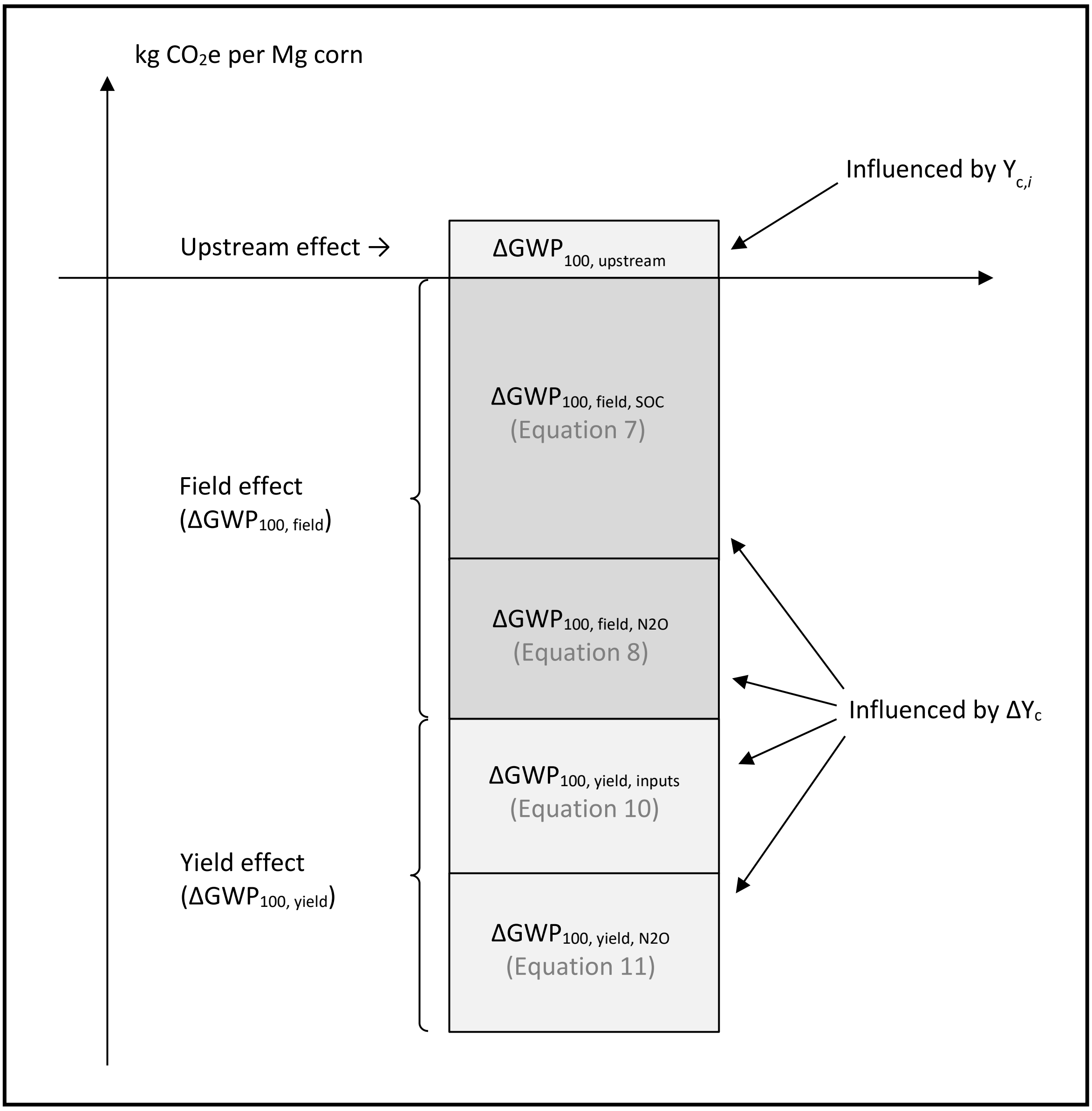

Fig. 4: Change in GHG emissions per Mg corn when using *P. bilaiae* (conceptual sketch, not to scale). For symbols used, see text.

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[30] Note that, since $GWP_{100,\ yield,\ N2O}$ expresses emissions from avoided N_2O, the value is negative.

By combination of Equation 1 and 2 (in Section 4.5) and Equation 6-11, the change in GHG emissions per Mg corn (when the *P.b.* inoculant is used) can be expressed as follows.

- $\Delta GWP_{100} =$

$$\Delta GWP_{100,\ inoculant,\ area} / Y_{c,i}$$

$$+ \Delta Y_c \cdot (GWP_{100,\ SOC} + GWP_{100,\ field,\ N2O} + GWP_{100,\ inputs} + GWP_{100,\ yield,\ N2O}) \qquad \text{(Equation 12)}$$

where $\Delta GWP_{100,\ inoculant,\ area}$ is the life cycle GHG emissions from use of the inoculant given per treated hectare (0.39 kg CO_2e ha^{-1}, cf. Equation 2)

Note that Equation 12 indicates a linear relation between yield increase and changes in GHG emissions. As discussed in Section 4.5, this approximation is reasonable as long as the formula is used with realistic (i.e. empirical) yield data.

As shown above, Equation 12 breaks down the GHG estimate to seven parameters. To conduct the uncertainty analysis for each of the four case studies, three things are required:

1. An absolute value for each parameter for each case study
2. A characterization of the distribution of each parameter (normal, triangular, etc.)
3. A relative uncertainty interval for each parameter

In relation to 1, $\Delta GWP_{100,\ inoculant,\ area}$, $Y_{c,i}$, and ΔY_c have already been quantified previously in the report (cf. Equation 12, Table 2, and Section 3.1.1, respectively). In order to derive absolute values for $GWP_{100,\ SOC}$, $GWP_{100,\ field,\ N2O}$, $GWP_{100,\ inputs}$, and $GWP_{100,\ yield,\ N2O}$, Equations 7, 8, 10, and 11 are used in combination with the GHG results in Section 4.2 and 4.3.

Calculation examples for continuous corn in Minnesota are shown below.

- $GWP_{100,\ SOC} = \Delta GWP_{100,\ field,\ SOC} / \Delta Y_c$
  $= -16.7 \text{ kg } CO_2\text{e } Mg^{-1} / 0.44 \text{ Mg } ha^{-1} = -38.0 \text{ kg } CO_2\text{e ha } Mg^{-2}$

  $\Delta GWP_{100,\ field,\ SOC}$ is found in Table 10 (Field effect, CO_2)

- $GWP_{100,\ field,\ N2O} = \Delta GWP_{100,\ field,\ N2O} / \Delta Y_c$
  $= -7.0 \text{ kg } CO_2\text{e } Mg^{-1} / 0.44 \text{ Mg } ha^{-1} = -15.9 \text{ kg } CO_2\text{e ha } Mg^{-2}$

  $\Delta GWP_{100,\ field,\ N2O}$ is found in Table 10 (Field effect, N_2O)

- $GWP_{100,\ inputs} = \Delta GWP_{100,\ yield,\ inputs} / \Delta Y_c$
  $= -6.6 \text{ kg } CO_2\text{e } Mg^{-1} / 0.44 \text{ Mg } ha^{-1} = -15.1 \text{ kg } CO_2\text{e ha } Mg^{-2}$

$\Delta GWP_{100,\text{ yield, inputs}}$ is found by summing GHG emissions from agricultural inputs[31] to 'Displaced corn' in Table 8.

- $GWP_{100,\text{ yield, N2O}} = \Delta GWP_{100,\text{ yield, N2O}} / \Delta Y_c$
  $= -6.06 \text{ kg } CO_2e \text{ Mg}^{-1} / 0.44 \text{ Mg ha}^{-1} = -13.8 \text{ kg } CO_2e \text{ ha Mg}^{-2}$

  $\Delta GWP_{100,\text{ yield, N2O}}$ is found in Table 8 (field emissions, N_2O, 'Displaced corn').

The absolute values for the seven parameters in Equation 12 can be found in Appendix E for continuous corn in Minnesota as well as for the three other case studies.

In the following, the uncertainty of each of the seven parameters has been assessed in terms of assumed distribution and relative confidence interval (95%).

- Life cycle GHG emissions from inoculant given per hectare ($\Delta GWP_{100,\text{ inoculant, area}}$): The largest uncertainty of the inoculant production relates to the disposal of organic waste (cf. Section 3.4). Since a worst-case estimate has been used in the LCA (where the organic waste is leading to methane emissions to the atmosphere), this estimate is assumed to make up the maximum value in the uncertainty interval. This interval is assumed to be triangular and left-skewed because flaring of landfill methane emissions could reduce the total emissions by 25% and collection and use of landfill methane could even off-set some of the other life cycle emissions from inoculant production and thereby reduce the footprint by 31% (calculations not shown). The flaring scenario has been used as the mode of the distribution and the 'reduction and use' scenario has been sued as the minimum of the distribution (values available in Appendix E). $\Delta GWP_{100,\text{ inoculant, area}}$ only has a small impact on the end results so the assumptions about distribution and uncertainty interval have little impact on the results of the uncertainty analysis.

- Initial corn yield per hectare ($Y_{c,i}$): This parameter is considered fixed (as the only one) in Equation 12, i.e. no uncertainty interval is assumed for $Y_{c,i}$.

- Yield increase per hectare (ΔY_c): The yield increase is assumed to follow a normal distribution and the confidence interval (95%) estimated by Leggett et al. (2015) has been applied in the uncertainty analysis (see Section 3.1.1). However, since the interval has only been provided with one significant digit for North Dakota, it has been assumed that the confidence interval for this state equals the central estimate ± two times the standard error, which give a CI95 interval of 0.22-0.52 (see Appendix E).

- CO_2 emissions from SOC per Mg corn per Mg change in yield ($GWP_{100,\text{ SOC}}$): This parameter is also assumed to follow a normal distribution and the relative confidence interval is assumed to be ±20%. This is based on Ogle et al. (2010).

- N_2O emissions (from inoculated field) per Mg corn per Mg change in yield ($GWP_{100,\text{ field, N2O}}$): This parameter is also assumed to follow a normal distribution

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[31] 'Inputs to the field'

and the relative confidence interval is assumed to be ±37%. This is based on Hutchinson et al. (2007).

- GHG emissions from displaced agricultural inputs per Mg corn per Mg change in yield ($GWP_{100, inputs}$): This parameter is also assumed to follow a normal distribution and the relative confidence interval is assumed to be ±20%. This is based on an uncertainty assessment of US corn conducted in SimaPro[32].

- N_2O emissions in displaced corn system per Mg corn per Mg change in yield ($GWP_{100, yield, N2O}$): The distribution and confidence interval for this parameter is assumed to be the same as for $GWP_{100, field, N2O}$, i.e. a normal distribution with a relative CI95 of ±37%. Note that there is likely some 'co-variance' between $GWP_{100, yield, N2O}$ and $GWP_{100, field, N2O}$ in the sense that, if N_2O emissions are under- or overestimated in the inoculant system, the same is likely to be true for the displaced corn system. This potential co-variance has not been factored into the uncertainty analysis, which means the estimated confidence intervals for the base case results are likely on the high side.

The relative uncertainty intervals listed above have been used to derive absolute uncertainty intervals for the parameters in Equation 12 for each of the four base case results ('Total change…') in Table 10 (see Appendix E). These uncertainty intervals and the assumed distribution for each parameter have been used as input to the statistical software tool JMP. With this software, 95% confidence intervals have been estimated for the base case results. These are shown in Fig. 5.

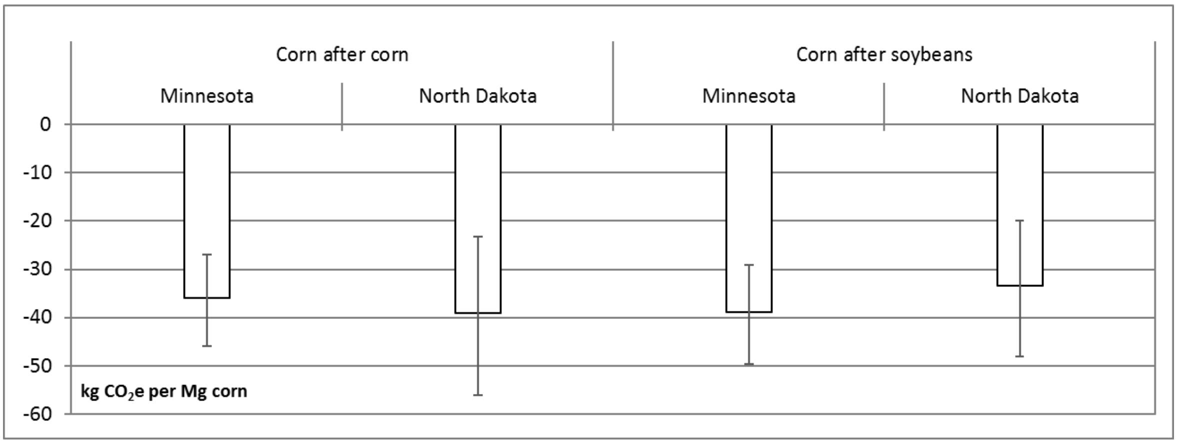

Fig. 5: Change in GHG emissions per Mg corn when using *P. bilaiae* (base case results) with estimated 95% confidence intervals (error bars)

As shown in Fig. 5, the confidence intervals are quite wide and clearly larger for North Dakota than for Minnesota. This is explained by the difference in relative confidence intervals for ΔY_c, which is ±23% for Minnesota and ±40% for North Dakota. A 50% reduction in the uncertainty related to ΔY_c would reduce the overall uncertainty estimates (CI95) by 33-44%.

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[32] SimaPro does not, at the time of writing, support uncertainty assessments of consequential processes so an attributional corn process was used (Maize grain {US}| production | Alloc Def, U). N_2O emissions were removed from the process as these are handled separately in the overall uncertainty assessment and the aim (in relation to $GWP_{100, inputs}$) was only to assess the uncertainty related to GHG emissions from displaced agricultural inputs.

In general, the wide uncertainty ranges can be explained by the large uncertainties associated with modelling of field emissions, i.e. CO_2 (from changes in SOC) and N_2O. Meanwhile, the confidence intervals in Fig. 5 may be over-estimated (as previously mentioned) because the analysis does not take into account the potential co-variance between N_2O from the inoculant system and N_2O emissions from the displaced corn system. In addition, there is some correlation between SOC and N_2O emissions, which has also not been considered in the uncertainty analysis. Never-the-less, the uncertainty analysis is considered to give a reasonable estimate of the uncertainty associated with the global warming results of each individual case study.

From Fig. 5 it is clear, that the mean of each result is within the CI of the other results. Hence, there is no statistical difference between the four base case results for the global warming impact category. Differences may in fact exist but, if so, they are covered by the uncertainty of the parameters in Equation 12.

6 Sensitivity analyses

This chapter explains four sensitivity analyses conducted to further explore the base case results.

6.1 *Varying time horizons for changes in SOC*

As previously discussed, the CO_2 field effect is time sensitive. The impact on GHG results of assuming different time horizons for changes in SOC (see Table 7) was therefore explored. Results are shown in Fig. 6 as changes in GHG emissions per Mg of corn produced with *P. bilaiae*. Note that the CO_2 field effect is separated out and all other changes (upstream effects, N_2O field effect, and the yield effect) have been shown together. Note also that the results for the 20-year time horizon for SOC are equal to the base case results in Table 10.

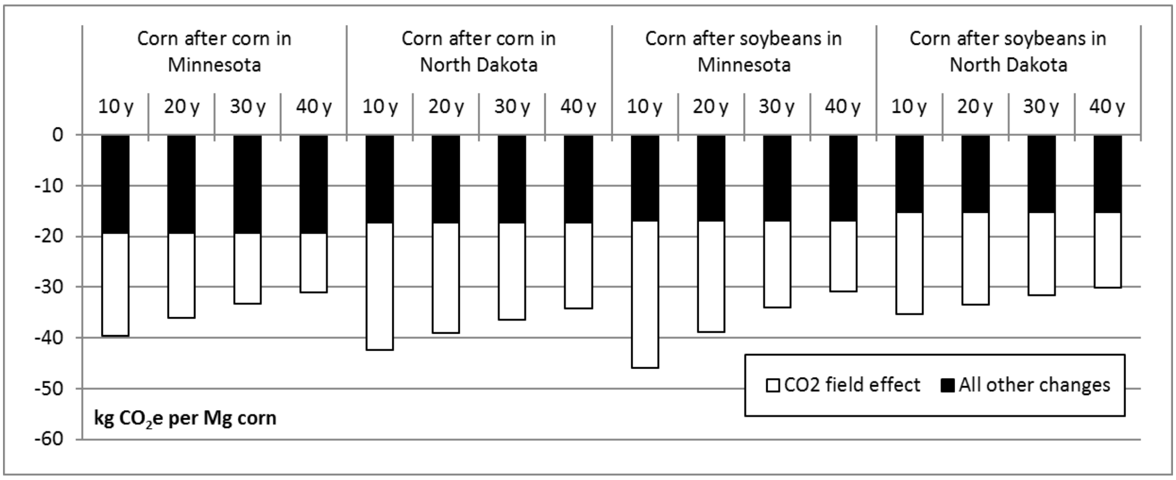

Fig. 6: Changes in life cycle greenhouse gas (GHG) emissions per Mg corn when using *P. bilaiae* based on different time perspectives for changes in SOC (soil organic carbon). Base case: 20 years

The average annual CO_2 field effect becomes less dominant the longer the time horizon (Fig. 6). The annual GHG reductions over a 40-year time perspective when applying *P. bilaiae* would amount to 10-11% (31-35 kg CO_2e per Mg corn) if continuous corn in the same state and with the same 40 y time perspective is used as the benchmark (~290 and ~300 kg CO_2e per Mg corn in Minnesota and North Dakota, respectively).

6.2 *Increased root fraction*

Some studies indicate that the use of *P. bilaiae* can increase the root fraction of crops (Gleddie 1993, Vessey and Heisinger 2001). The potential impact on life cycle GHG emissions was investigated by calibrating the DayCent model to simulate five and ten percent increased root fractions with *P. bilaiae* while maintaining the same yield increase as in the base case. Results are shown in Fig. 7. Note that the results for zero percent change in root fraction (unchanged root-to-shoot ratio) are equal to the base case results in Table 10.

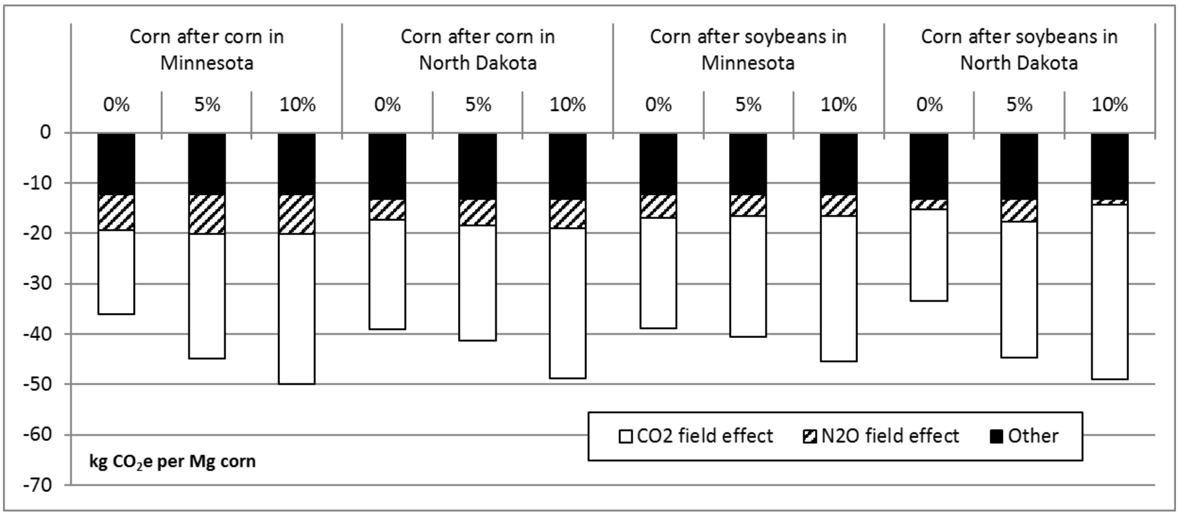

Fig. 7: Changes in life cycle greenhouse gas (GHG) emissions per Mg corn when using *P. bilaiae* assuming increases in root fraction of respectively 0% (base case), 5%, and 10%

The N_2O emissions were more or less unaffected by increased root fraction, with the exception being corn after soybean in North Dakota where there is a fine trade-off between increased carbon inputs from roots (more dissolved organic carbon being available for increased N_2O emissions) and increased water uptake to the roots (which can decrease N_2O emissions). As for the CO_2 field effect, the larger root fraction leads to increased soil carbon inputs and thereby larger reductions in CO_2 emissions.

6.3 Different approach for modeling of CO_2 from changes in SOC

The approach taken in the base case analysis to convert SOC changes (as modeled in DayCent) into an equivalent CO_2 emission (cf. description of DayCent simulations in the Methodology section) is consistent with IPCC methodology, the European Renewable Energy Directive, and other LCA studies in the literature. However, this 20-year amortization approach is not consistent with the GWP100 approach (the common metric for GHG emissions), which is based on radiative forcing over a 100-year time period (Ramaswamy et al. 2001).

We therefore explored another method published by Petersen et al. (2013) to convert changes in SOC into equivalent CO_2 emissions. The basic idea is to look at the change in radiative forcing related to a single event with impact on SOC. In this case, the event would be one application of *P. bilaiae* in corn production. The *P. bilaiae* application impacts the temporal development of SOC over the subsequent time period. This development can be compared to the reference scenario (baseline). By converting the differences in SOC into radiative forcing, the global warming potential (GWP) can be determined for any given accounting period. Following this approach, one year of corn production with *P. bilaiae* (one application) and 99 years of corn production without *P. bilaiae* was first modeled (in DayCent). As a reference (baseline), 100 years of corn production without *P. bilaiae* was modeled (also in DayCent). This allowed for tracking of the difference in SOC between the inoculant system (one *P.b.* application in year 1) and the reference system, which in turn allowed us to derive a GWP100 for the CO_2 field effect of *P. bilaiae*. Note that this approach is not dependent on the selection of a certain use period for *P. bilaiae* as opposed to the 'annualization approach' applied in the base case analysis (cf. Fig. 6). Instead, the CO_2 soil effects can be viewed in isolation for one year of corn production (as with all the other elements of the analysis). A GWP100 of -12.9 g CO_2e per m^2 y for continuous corn in Minnesota was thereby estimated (details available in

Appendix C). When multiplied with the area treated with *P. bilaiae* per functional unit (inverse of corn yield in reference system), this gives a CO_2 soil effect of -12 kg CO_2e per Mg corn. Results for all investigated scenarios are available in Table 13.

Table 13: Changes in life cycle greenhouse gas (GHG) emissions with the use of *P. bilaiae* (*P.b.*) on corn (kg CO_2e per Mg corn) with CO_2 soil effect based on method by Petersen et al. (2013)

	Continuous corn		Corn after soybean	
	Minnesota	N. Dakota	Minnesota	N. Dakota
Upstream effect (*P.b.* production)	0.04	0.05	0.04	0.05
Field effect, CO_2†, 20 y avg.	-12.0	-19.1	-12.6	-20.1
Field effect, N_2O	-7.0	-4.1	-4.6	-1.9
Yield effect (displacement)	-12.7	-13.7	-12.7	-13.7
Total change with *P. bilaiae* on corn	-31.7	-36.8	-29.9	-35.7
Continuous corn without *P.b.* (benchmark)	258.0	270.6	258.0	270.6
Reduction with *P.b.* versus benchmark	12.3%	13.6%	11.6%	13.2%

† From change in SOC (soil organic carbon)

As shown in Table 13, the estimated reduction in GHG emissions from the use of *P. bilaiae* decreases somewhat when applying the method by Petersen et al. (2013) as compared to the standard 20 y average approach (Table 10). As also shown, the CO_2 field effect is higher in North Dakota than in Minnesota. This is mainly explained by climatic differences between the two states.

It should be mentioned that Petersen et al. (2013) recommend using soil C estimates to 1 m in depth. Meanwhile, DayCent cannot estimate SOC change past 20 cm. For many soils, 20 cm is sufficient as tillage often does not reach this depth (i.e. western prairie soils). For the case studies in the present LCA where tillage is consistent between both treatments (with and without the use of the *P.b.* inoculant), SOC changes occurring beneath 20 cm are considered to be irrelevant for the end results of the LCA.

6.4 *Adding estimate of GHG impact from ILUC*

As previously discussed, the yield increasing effect of *P. bilaiae* reduces the need for crop production elsewhere. For the base case (cf. Table 10), this has been modeled by assuming displacement of inputs to corn production without *P. bilaiae* in another location, including displacement of the associated emissions. However, the implications of reduced pressure on land use have not been modeled (up until now). In other words, the (indirect) land use impact of reducing corn production elsewhere (cf. Fig. 1 and 2) has not been considered. The discussion about indirect impacts on global land use from changes in the supply and demand for crops has garnered much attention in the debate about so-called first generation biofuels, e.g. bioethanol from corn. Meanwhile, this discussion has been focused on land that might be converted from natural vegetation to agriculture as an (indirect) result of increased crop demand resulting from biofuels production (Hertel et al., 2010; Valin et al., 2015). In the current sensitivity analysis, focus is on the opposite effect, i.e. land that may not be converted to agriculture due to an increase in supply from existing cropland[33].

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[33] The global agricultural area (as a whole) is still expanding. If yields can be raised on existing croplands, some of the ongoing expansion can (indirectly) be prevented. For an in depth discussion, see Kløverpris and Mueller (2013).

Many of the studies conducted on indirect land use change (ILUC) from biofuels are based on economic equilibrium models with a medium-term time perspective. As a result, they consider three effects of a change in crop demand (or supply): Change in area of cultivated land, change in intensity of crop production (fertilizer use, etc.), and change in use patterns of crops due to changes in crop prices. The last-mentioned effect (change in use patterns) is usually not considered in LCA because the methodology is based on a longer-term time perspective wherefore the supply of goods and services is assumed to be fully elastic, i.e. an increase in demand will be met by a corresponding (1:1) increase in supply (Weidema et al. 2013). Other ILUC models (not based on economic equilibrium models) have also been proposed (De Rosa et al., 2016). One of these models was published by Schmidt et al. (2015). It applies the longer-term time perspective (which is typical for LCA) and hence was applicable to the present LCA study. Here, the model by Schmidt et al. (2015) was used to estimate the ILUC effect (in terms of GHG emissions) of *P. bilaiae* on corn.

According to the ILUC model developed by Schmidt et al. (2015), the occupation of 1 hectare of average US cropland for one year results in an indirect climate effect corresponding to a GHG emission of 2050 kg CO_2. This effect is comprised of two elements. The first is the indirect impact on ongoing global land conversion. By occupying existing cropland, land conversion elsewhere will occur sooner than it would otherwise and so will the resulting GHG emissions from decomposition of natural vegetation. In addition, farmers will be incentivized to intensify production when more land is occupied, e.g. by use of more fertilizers, which is also associated with GHG emissions.

In the opposite case, if the supply from existing cropland is increased, it will delay ongoing conversion of natural vegetation and reduce the intensity of crop production elsewhere. As shown in Fig 1 and 2, an area of cropland (elsewhere) is ‘released’ when 1 Mg corn is produced with *P. bilaiae* (assuming fixed production at the given time). In the Minnesota case, the area released is 38 m^2 [1 / (10.7 Mg ha^{-1}) · (0.44 Mg ha^{-1}) / (10.7 Mg ha^{-1}) · 10,000 m^2 ha^{-1}] and, for North Dakota, it is 51 m^2 [1 / (8.5 Mg ha^{-1}) · (0.37 Mg ha^{-1}) / (8.5 Mg ha^{-1}) · 10,000 m^2 ha^{-1}][34]. As corn production effectively occupies the land for the full year, the land occupation saved with *P. bilaiae* per Mg of corn is 38 and 51 m^2·year for Minnesota and North Dakota, respectively. These land savings are multiplied with the estimated ILUC impact from occupation of average US cropland (2050 kg CO_2e ha^{-1} y^{-1}) based on Schmidt et al. (2015). An estimate of the ILUC effect from *P. bilaiae* is thereby obtained. Results are shown in Table 14 where the estimated ILUC effect is added to the remaining results. Note that it is the CO_2 field effect based on the GWP method by Petersen et al. (2013), which is used in Table 14. This is because the same method is built into the ILUC model by Schmidt et al. (2015). Hence, these two approaches are combined for consistency.

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[34] The area released (saved land occupation) is calculated as the land occupation in the reference system multipied by the relative change in land occupation (per Mg corn) when *P.b.* is introduced. The land occupation in the reference system can be expressed as the inverse of the initial corn yield (1 / $Y_{c,i}$). The relative change (reduction) in land occupation (per Mg corn) can be expressed as the area displaced in the inoculant system (B in Fig. 1) divided by the area cultivated in the reference system (A in Fig. 1). This is equal to ΔY_c / $Y_{c,i}$ (see last part of Section 2.2.6.1). Hence, the area released can be calculated as (1 / $Y_{c,i}$) multiplied with (ΔY_c / $Y_{c,i}$). This is the formula that has been used in the text above (and then hectares have been converted to square meters).

Table 14: Changes in life cycle greenhouse gas (GHG) emissions with the use of *P. bilaiae* (*P.b.*) on corn (kg CO_2e per Mg corn) when estimate of indirect land use change (ILUC) is included

	Continuous corn		Corn after soybeans	
	Minnesota	N. Dakota	Minnesota	N. Dakota
Upstream effect (*P.b.* production)	0.04	0.05	0.04	0.05
Field effect, CO_2†, GWP100	-12.0	-19.1	-12.6	-20.1
Field effect, N_2O	-7.0	-4.1	-4.6	-1.9
Yield effect (displacement)	-12.7	-13.7	-12.7	-13.7
ILUC (incl. intensification)	-7.9	-10.5	-7.9	-10.5
Total change with *P.b.* on corn	-39.6	-47.3	-37.8	-46.2
Continuous corn without *P.b.* (benchmark)	258.0	270.6	258.0	270.6
Reduction with *P.b.* versus benchmark	15.3%	17.5%	14.6%	17.1%

† From change in SOC (soil organic carbon) – based on approach by Petersen et al. (2013)

Adding the estimated ILUC effect increases the relative GHG reduction per Mg of corn produced with *P.b.* to around 15-17% of which the estimated ILUC effect accounts for 3-4 percentage points. Here, it is important to keep in mind that several ILUC models exist and that ILUC modeling is still a research field in development. Hence, results might have looked different with another model and should therefore be interpreted with caution.

It is furthermore important to understand that the ILUC modeling *first* assumes that nearby corn land is ‘freed up’ (38 m^2y in the case of Minnesota) and *then* considers the market-mediated ILUC effects of this. This means that the *P.b.* inoculant is first credited for displacing corn production elsewhere (as in the base case) and then gets an extra credit for avoiding ILUC. An equally valid approach would have been to consider the change in crop production from existing land (i.e. the increased output of corn caused by *P. bilaiae*) and its *direct* impact on global crop production in terms of land use and crop production intensity (i.e. without first assuming the intermediate ‘corn displacement step’ before estimating ILUC). The latter approach would effectively collapse the yield effect and the ILUC effect into one category, expressing the overall market response to a change in crop supply in terms of land use change and crop production intensity. Results would be the same as in Table 14, except the Yield effect should be subtracted (now solely made up by the ILUC effect).

A short additional discussion of other ILUC models has been added in Appendix G.

6.5 Changing assumptions regarding displaced corn

In the base case analysis, it was assumed that conventional corn without *P. bilaiae* (within the same state) would be displaced by the yield increase obtained with the *P.b.* inoculant. This assumption has implications for the yield effect. In the present sensitivity analysis, it has been investigated how results would be impacted if other types of corn production (with different life cycle inventories) were assumed to be displaced. The following corn production processes have been selected from the ecoinvent database.

- Maize grain {US}| production | Conseq, U
- Maize grain {RoW}| production | Conseq, U
- Maize grain, Swiss integrated production {CH}| production | Conseq, U
- Maize grain organic {CH}| production | Conseq, U
- Maize grain organic {RoW}| production | Conseq, U

Equation 4 has been used to assess the yield effect for global warming on the basis of above-mentioned processes. Hence, drying and transport processes have been removed from the processes.

The principle outlined in Equation 4 for global warming has also been applied to the other considered impact categories. Results have been shown in a table in Appendix F where impact on the yield effect and total change in environmental impacts has been shown.

Appendix F also contains a graph where the total change in environmental impacts in the base case has been shown as index 100. The total change in environmental impacts when changing assumptions regarding displaced corn has also been indexed in relation to the base case and shown in the same graph.

The global warming category is the least sensitive to assumptions regarding displaced corn. This is partly because the total change in global warming is governed by the yield effect as well as the field effect. With the selected alternative corn processes (see list above), reductions in GHG emissions (contributions to global warming) could be up to 12% higher[35] than in the base case. None of the alternatives for displaced corn production gives a lower estimate of GHG savings than in the base case, thereby indicating that the present LCA study is being conservative in the approach to the modeling of the yield effect in terms of global warming (rather underestimating than overestimating the benefits of the *P.b.* inoculant).

The same can be said about the eutrophication impact category. The alternative assumptions about displaced corn all give higher savings of nutrients to the environment, as much as 86% (corn after soy in Minnesota, assuming displacement of organic corn from ‘Rest of the World’). This indicates that the approach to the yield effect also gives conservative results for the eutrophication impact category.

As for the remaining four impact categories (fossil energy, photochemical ozone formation, acidification, and land occupation), the picture is more blurred. These impacts are (almost[36]) entirely governed by the yield effect and therefore more sensitive to the

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[35] Corn after soy in North Dakota assuming displacement of Swiss organic corn

[36] The upstream effect also has a small influence

assumptions regarding displaced corn. These impacts are also those with the lowest relative change (cf. Table 11).

Savings in fossil energy could be twice as high as in the base (Minnesota cases with displacement of conventional corn from ‘Rest of the World’) but they could also be 34% lower (corn after corn in North Dakota, assuming displacement of Swiss organic corn). Savings in contributions to photochemical ozone formation shows almost the same picture, although with a slightly larger span (from -32% to +111%).

Higher savings in acidification impacts are found with all alternative assumptions regarding displaced corn, ranging from 7% higher savings compared to the base case (assuming displacement of conventional US corn) to as much as 330% higher savings (assuming displacement of organic corn from rest of the world).

Savings in land occupation could be slightly lower (~6%) in North Dakota (assuming displacement of conventional corn from the US or ‘Rest of the World’) or substantially higher (up to 163%) if organic corn (Swiss or ‘Rest of the World’) was assumed to be displaced. This is explained by the lower yield obtained in organic agriculture as compared to conventional.

All in all, the present sensitivity analysis shows that results of the present LCA are sensitive to assumptions regarding displaced corn, especially for fossil energy sources, photochemical ozone formation, and land occupation. Meanwhile, results are quite stable for the global warming category (2-12% increase in saved GHG emissions per Mg corn). The sensitivity analysis also shows that the present LCA study is generally conservative in the sense that it does not overestimate the benefit of the yield effect.

Finally, some of the most extreme variations are seen when assuming displacement of organic corn. While it may be interesting to investigate this, it is also important to consider whether additional conventional corn produced via *Penicillium bilaiae* would displace organic corn. Likely conventional and organic corn are traded on different markets and do not, in reality, displace each other. Hence, one should be careful when interpreting the results assuming displacement of organic corn.

7 Alternative scenario: Ethanol from additional corn

Due to the challenges of determining the impacted crops and land use by increased corn production with *P. bilaiae*, a scenario was investigated where the additional corn produced with *P. bilaiae* was converted into ethanol and used in transportation to replace gasoline. A 'well-to-wheel' study by Wang et al. (2012) was used as the basis for estimating GHG emissions from corn ethanol. Wang et al. (2012) estimated a carbon footprint of 62 g CO_2e MJ^{-1} of which 40 g CO_2e MJ^{-1} came from corn production and land-use change. Hence, if corn ethanol was made from the yield increase obtained with *P. bilaiae* (with no additional use of land and inputs, except the inoculant), the ethanol would have a carbon footprint of 22 g CO_2e per MJ ethanol [(62-40) g CO_2e MJ^{-1}]. On the basis of Ecofys (2014), the replacement of gasoline with ethanol would ultimately prevent GHG emissions of 115 g CO_2e per MJ. With these numbers, the results shown in Table 15 were derived. Note that the ethanol production and the resulting gasoline replacement is referred to as the 'Yield effect via ethanol production'[37].

Table 15: Change in life cycle greenhouse gas (GHG) emissions with the use of *P. bilaiae* (*P.b.*) on corn (kg CO_2e per Mg corn) assuming additional yield is used for ethanol production to displace gasoline

	Continuous corn		Corn after soy	
	Minnesota	N. Dakota	Minnesota	N. Dakota
Upstream effect (*P.b.* production)	0.04	0.05	0.04	0.05
Field effect (20 y avg. for CO_2)	-23.7	-25.9	-26.6	-20.2
Yield effect via ethanol production†	-34.5	-36.5	-34.5	-36.5
Total change with *P.b.* on corn	-58.1	-62.3	-61.0	-56.6
Continuous corn w/o *P.b.* (benchmark)	258.0	270.6	258.0	270.6
Reduction w. *P.b.* compared to benchmark	22.5%	23.0%	23.6%	20.9%

† Assumed ethanol yield: 425 l Mg^{-1} corn (Wang et al. 2012)

As shown in Table 15, the use of the yield increase from *P. bilaiae* to produce ethanol and thereby replace gasoline gives the most attractive result (out of the options investigated in the present study) in terms of global warming with 21-24% reduction in the impact compared to the benchmark (1 Mg continuous corn). However, the use of additional yield for ethanol does not necessarily further improve results in the other impact categories (quantitative analysis not conducted). It should also be mentioned that, in case of a higher ILUC factor in Section 6.4, the yield increase from *P. bilaiae* might have been better used to relieve pressure on agricultural land than to displace gasoline through ethanol production. It thereby also follows that, with a lower ILUC factor in Section 6.4, it would seem even more beneficial (in terms of global warming) to use additional corn from existing cropland for ethanol production. To get a sense of the ILUC results derived by the ILUC model by Schmidt et al. (2015) in comparison to other ILUC results, see Appendix G.

Note that the yield effect via ethanol (see Table 15) is not the same in Minnesota and North Dakota. This is because the yield increase is not the same (cf. Section 3.1.1) but also because the yield effect (via ethanol) is assigned as a co-product credit to the amount of corn, which does not exceed the yield with the *P.b.* inoculant, i.e. 10.7 Mg ha^{-1} in

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[37] Calculation example for continuous corn in Minnesota: 0.44 Mg ha^{-1} / 10.7 Mg ha^{-1} · 425 l Mg^{-1} · 21.2 MJ l^{-1} · (22-115) g CO_2e MJ^{-1} = -34,457 g CO_2e Mg^{-1} = -34.5 kg CO_2e Mg^{-1}

Minnesota and 8.5 Mg ha^{-1} in North Dakota. Hence, the ethanol co-product depends on both the yield increase and the initial corn yield (before use of the inoculant).

An interesting note regarding the use of additional corn yield for ethanol production, is that the Roundtable on Sustainable Biomaterials (RSB) has developed a certification standard for biofuels produced from feedstocks with no or low ILUC risk, including feedstocks from ‘above baseline yield’ (RSB 2015).

8 Interpretation

This section gives some broader considerations to the results of the LCA and summarizes the main findings.

8.1 Conclusions

The yield increasing effect of *P. bilaiae* on US corn production is accompanied by a number of environmental benefits with no observed net trade-offs within the impact categories studied. The application of *P. bilaiae* leads to higher soil carbon sequestration and reduced emissions of nitrous oxide (the field effect). In addition, the extra yield obtained with *P. bilaiae* can displace crop production elsewhere and thereby reduce environmental pressures (the yield effect). In the base case, GHG savings per Mg of US corn produced with *P. bilaiae* ranged from 34 to 40 kg CO_2e with an average of 37 kg CO_2e (SOC changes annualized over 20 years). If compared to the GHG emissions from conventional continuous corn production, the GHG savings from *P. bilaiae* correspond to 13-15%. Over a 40-year time perspective, changes in SOC are less dominant and the GHG savings fall to 31-35 kg CO_2e per Mg corn (10-11% compared to benchmark). The potential increase in crop root fraction from *P. bilaiae* could increase soil carbon sequestration even further than in the base case resulting in GHG savings of up to 50 kg CO_2e per Mg corn (10% increase in root fraction, SOC changes annualized over 20 years).

As for the CO_2 field effect, a different approach for the conversion of SOC changes to equivalent CO_2 emissions (not based on annualization and more in line with the GWP concept) reduced the GHG benefit of *P. bilaiae* slightly. Meanwhile, the estimated GHG emissions from 1 Mg corn with *P. bilaiae* were still 12-14% lower than the benchmark (continuous corn without *P. bilaiae*).

Adding considerations about indirect land use change (in combination with the GWP-based modeling of the CO_2 field effect) improved the estimated GHG reductions obtained with *P. bilaiae* on corn to a level which was 15-17% better than the benchmark.

The different sensitivity analyses indicate that results are quite robust and that GHG improvements for *P.b.* on corn in Minnesota and North Dakota lie in the range of 10-17%.

However, all of the results are subject to the underlying uncertainty related to the yield increase data published by Leggett et al. (2015), the emissions modeling in DayCent, and the modeling of displaced corn production. Based on a quantitative uncertainty analysis, the relative 95% confidence intervals of the base case results for global warming are -28% / +25% for Minnesota and -43% / +41% for North Dakota. The difference between the two states is explained by the higher uncertainty related to the yield increase data or North Dakota. The uncertainty intervals may be slightly overestimated because some co-variance between field emissions has not been captured in the analysis. Results for other impact categories are expected to be less uncertain because they are less influenced by the (inherently uncertain) modeling of field emissions in DayCent.

Finally, it was found that the use of the additional corn yield obtained with *P. bilaiae* for ethanol production (to replace gasoline) could increase GHG savings to 57-62 kg CO_2e per Mg corn (21-24% better than benchmark). However, this would reduce the benefits in other impact categories (as compared to the base cases).

8.2 Perspectives

The US has an annual production of roughly 350 million Mg of corn (FAOSTAT 2016) on which *P. bilaiae* could be applied. Meanwhile, the inoculant will not necessarily give the same response as observed in Minnesota and North Dakota on all corn fields. Novozymes has publicly stated that a general yield increase of more than 3 bushels per acre (>0.19 Mg ha^{-1}) can be expected. This is based on recent extensive field trials that have not (at this point in time) been published in the peer-reviewed literature. The measured general yield increase applies to responsive fields. A conservative estimate of the share of responsive corn fields in the US is 60% (Burns 2017).

Based on total annual corn production in the US, the assumed response rate for *P.b.* on corn as well as general LCI data for US corn production in the ecoinvent database, it is estimated that *P. bilaiae* could provide a total GHG reduction of roughly 3.9 million Mg CO_2e y^{-1} if applied on all US corn fields (details in Appendix D). That is equivalent to taking 820,000 US passenger cars off the road, assuming annual vehicle emissions of 4.7 Mg CO_2e per car (US EPA, 2016). Note that the estimation implicitly assumes that additional corn (obtained via *P. bilaiae* from responsive corn fields) replaces corn from non-responsive corn fields.

Had the ILUC results been applied (assuming reduced land conversion globally), the total estimated potential would have been 4.5 million Mg CO_2e y^{-1} (calculations not shown).

In a world where reductions in greenhouse emissions are becoming ever more important, one could imagine that the climate benefits of microbial inoculants could become relevant within new or existing carbon trading schemes.

8.3 Recommendations

To improve the present LCA further, additional research could be conducted on the following aspects:

- As shown in one of the sensitivity analyses, potential increase in the root fraction of crops treated with *P. bilaiae* could have a substantial impact on the LCA. However, this potential effect is not yet fully documented. More research in this field is hence encouraged.

- There are also indications that the use of *P. bilaiae* can lead to lower harvest moisture in corn (not discussed previously in the report). This could reduce energy required for drying and thereby have a downstream effect. Hence, further exploration of this potential effect is also encouraged.

- Crop systems were modeled in DayCent using the corn yield data from Leggett et al. (2015) listed in Table 2 and the yield deviations listed in Table 3.1.1. As mentioned in Section 3.1.1, the average yield of corn after soybeans modeled in DayCent did not match the yield deviations from Leggett et al. (2015) exactly (5-7% discrepancy). This creates a slight inconsistency between the yield effect and the field effect for corn after soybeans. It means that (for the corn-soybean rotation) the yield effect for Minnesota has been slightly underestimated whereas the yield effect for North Dakota has been slightly overestimated. This is not deemed to have any significant impact on the overall conclusions of the study but, if the research were to be improved, the DayCent modeling of the corn-soybeans rotations could be fine-tuned to match the reported yield deviations exactly[38].

- Further research related to the DayCent modeling could also focus on the potential impact of different assumptions regarding land use history of the fields where the inoculant is applied.

- Finally, the present study indicates that widespread use of the *P.b.* inoculant (on all US corn) will be beneficial from an environmental point of view. Even if the inoculant is applied on non-responsive fields, the benefits accruing from responsive fields will vastly make up for the upstream effect on non-responsive fields (cf. Appendix D).

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[38] On the other hand, fine-tuning to match site conditions exactly could also lead to over-calibration, which could lower the general representativeness of results and thereby reduce validity when upscaling to regional or national level.

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10 Appendix A: Cross-checking of field trial fertilizer data

This appendix discusses the applied fertilizer data in Table 2 in relation to values from the literature.

For corn-after-corn in Minnesota, the University of Minnesota (Rehm et al., 2006) has an acceptable range of roughly 110-200 kg N ha^{-1} for soils considered highly productive, depending on the ratio between N price and crop value. The data in Table 2 (181 kg N ha^{-1}) is within that range. For corn after soy in Minnesota, the same acceptable range is roughly 80-160 kg N ha^{-1} (Rehm et al., 2006). The data in Table 2 (158 kg N ha^{-1}) is in the high end of this range, which may indicate that some farmers tend to use more N fertilizer on corn-after-soy than recommended.

The average N use (regardless of previous crop) for North Dakota (based on the 19 trials for which N data were available[39]) was 138 kg N ha^{-1}. For corn, North Dakota State University is recommending 135-200 kg N ha^{-1} incl. soil N for a yield potential of 6.3-9.4 Mg ha^{-1} (Franzen, 2010). Assuming a straight-line correlation, a yield of 8.5 Mg ha^{-1} (the average yield without *P. bilaiae* for the North Dakota field trials, see Table 2) would correspond to a recommendation of 182 kg N ha^{-1} (incl. soil N). Based on five North Dakota field trial reports[40], we estimate average soil N around 34 kg ha^{-1}, which gives an estimated fertilizer requirement around 148 kg N ha^{-1} (182 kg N ha^{-1} – 34 kg N ha^{-1}). This number has been used for corn-after-corn in the LCA (see Table 2). Note that it is above the average of 138 kg N ha^{-1} not considering previous crop (as it should be). For corn-after-soybean, Franzen (2010) recommends a nitrogen credit of 45 kg N ha^{-1}. We therefore used 103 kg N ha^{-1} for this case in the LCA (see Table 2). It is acknowledged that we thereby ignored some farmers' potential over-use of nitrogen on corn-after-soy as compared to the recommendations (cf. discussion of Minnesota data above). This may have led to an underestimation of the fertilizer saving obtained with *P. bilaiae* per unit of corn produced because this saving is a percentage of the overall input of N (determined by the relative yield increase).

As for phosphorus, the Minnesota data in Table 2 (Total P) corresponds to the (broadcasting) recommendations for a low soil test (Rehm et al., 2006). The potassium data for Minnesota in Table 2 (Total K) is close to the ecoinvent number while it corresponds to a high soil test for North Dakota (Franzen, 2010). The phosphorus data in Table 2 for North Dakota is slightly below the ecoinvent number and corresponds to the (broadcasting) recommendations for a low to medium soil test (Franzen, 2010). All in all, we find that the fertilizer data in Table 2 appears reasonable. Phosphorus use in the Minnesota scenario may be on the high side while potassium use in the North Dakota scenario may be on the low side. Both aspects turn out to have little influence on the results of the LCA.

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[39] Not counting three outliers below 10 kg N ha^{-1}

[40] Part of the background information compiled and published by Leggett et al. (2015) and made available to the authors by Leggett (2012).

11 Appendix B: Nitrogen mass balances

The table below shows a detailed overview of the nitrogen flows modeled in DayCent.

Nitrogen balances (kg N ha^{-1}) – 40 year averages. The sum of each column is not zero exactly due to rounding errors.

		Minnesota Corn-corn		Minnesota Corn-soybeans		North Dakota Corn-corn		North Dakota Corn-soybeans	
		Ref.[a]	*P.b.*[b]	Ref.[a]	*P.b.*[b]	Ref.[a]	*P.b.*[b]	Ref.[a]	*P.b.*[b]
Input	N fertilizers[c]	181.0	181.0	84.0	84.0	148.0	148.0	56.5	56.5
	Symbiotic N fixation	-	-	71.9	72.5	-	-	102.4	103.1
	Atmospheric/non-symbiotic N	8.0	8.0	7.8	7.8	6.8	6.8	6.8	6.8
Output	N in grain	110.9	117.0	129.0	130.7	90.8	95.1	106.6	108.4
	Dinitrogen oxide (N_2O) to air, as N	3.4	3.2	2.4	2.4	2.6	2.5	2.2	2.2
	Nitrogen oxides (NO_x) to air, as N	6.8	6.5	4.7	4.6	4.4	4.2	3.7	3.7
	N_2 to air	0.4	0.4	0.3	0.3	0.2	0.2	0.2	0.2
	Ammonia (NH_3) to air, as N	5.9	6.3	7.4	7.5	4.9	5.2	6.8	6.9
	Nitrogen leaching, mineral	37.5	29.9	24.7	22.6	10.8	6.8	29.2	27.6
	Nitrogen leaching, organic	1.5	1.5	0.9	0.9	1.0	1.0	0.7	0.7
Stock	Mineral nitrogen	1.1	0.9	0.5	0.5	14.6	10.9	10.3	10.0
Changes	Organic nitrogen	20.7	22.1	-7.0	-6.1	25.4	28.3	4.9	5.6

[a] Reference system without the use of *P. bilaiae*

[b] System with use of *P. bilaiae* on corn

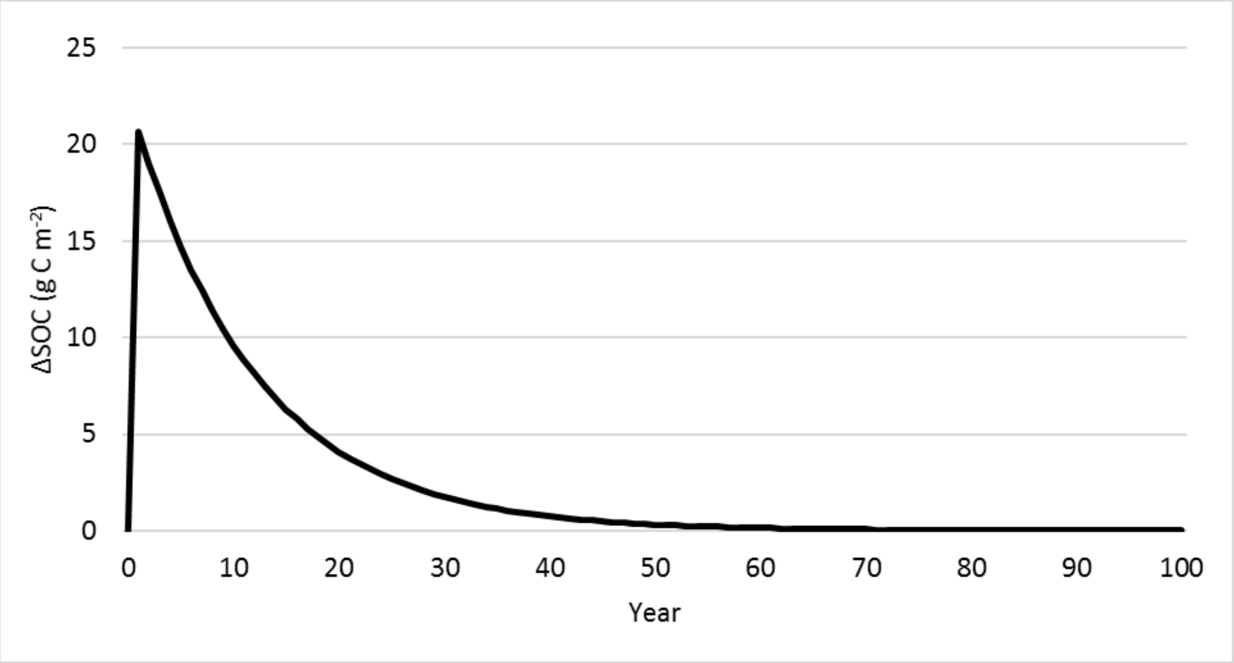
[c] N fertilizers added throughout one full crop rotation (i.e. the corn-soybean rotations contain N fertilizer for corn *and* soybeans)

Note that the table above contains annual averages for the full crop rotations. Hence, the input of N fertilizer to the corn-soy rotations is substantially lower than the input of N fertilizer to corn after soybeans shown in Table 2. This is because soybeans receive a much lower input of N fertilizers (10 kg N ha^{-1}) than corn (due to the symbiotic N fixation in soybean production).

12 Appendix C: Alternative CO_2 modeling from SOC

Modeling of one year of *P. bilaiae* application was facilitated in DayCent by introducing a single year of *P.b.* use and comparing it to previous baseline scenarios (without *P.b.*) for continuous corn and corn after soybeans in Minnesota and North Dakota. As DayCent does not have the capability to model the impact of a single year of *P.b.* application on the availability of labile P, this effect was instead represented by using a corn cultivar that captured the additional crop carbon inputs tied to the observed increase in yield (only for year 1) as a result of the *P.b.* inoculant. The simulations were modeled under a business-as-usual scenario (historical climate) for a 100-year timespan to quantify the difference in the rate of SOC change between the reference and inoculant systems. R statistical software (R core team, 2015) was used to apply a best-fit model using an exponential decay function similar to the one used by VandenBygaart et al. (2008) for each of the crop systems and locations to derive the difference in the rate of SOC change.

The figure below shows the difference between a continuous corn system where *P. bilaiae* has been applied in year 1 and a similar system where it has not been applied (modeled in DayCent).



Difference in soil organic carbon (SOC) between continuous corn system with application of *P. bilaiae* in year 1 and reference system (no use of *P. bilaiae*)

As the figure shows, the difference in SOC between the two systems after the first year is 21 g C m^{-2}. This is explained by the relative increase in carbon sequestration obtained with *P. bilaiae* in year 1 (resulting in a larger yield and increased crop carbon inputs as compared to the reference system). This corresponds to a negative CO_2 emission in year 1. Meanwhile, the difference in SOC between the two systems is subsequently reduced until it is close to zero after roughly 60 years. This is because there is more carbon in the soil in the inoculant system (due to the application of *P. bilaiae* in year 1) and therefore more carbon is oxidized and released to the atmosphere as CO_2. This corresponds to net CO_2 emissions from year 2 to around year 60. Each year's CO_2 emission can be converted to a

‘GWP contributor’, which relates to the use of *P. bilaiae* in year 1. The conversion from a CO_2 emission in a given year to its contribution to the GWP takes place by a characterization factor, which reflects the decay of CO_2 in the atmosphere as well as the contribution to global warming within the relevant accounting period (Petersen et al. 2013). In this case, the GWP period is 100 years counting from the application of *P. bilaiae* in year 1. By summing up contributions to GWP from all years in the accounting period, the GWP100 for the impact on SOC is obtained. In the case of continuous corn in Minnesota, the GWP100 for the change in SOC is -12.9 g CO_2e m^{-2} y^{-1} (see table below for details).

Estimation of GWP100 for the impact on SOC when applying *P. bilaiae* on continuous corn in Minnesota

Year	ΔSOC g C m^{-2}	Emission g CO_2 m^{-2}	Characterization factor[a]	GWP100 g CO_2e m^{-2}
1	20.684	-75.842	1.00	-75.84
2	18.996	6.191	0.98	6.07
3	17.445	5.686	0.96	5.48
4	16.021	5.222	0.95	4.94
5	14.713	4.795	0.93	4.47
6	13.512	4.404	0.92	4.03
7	12.409	4.044	0.90	3.65
8	11.396	3.714	0.89	3.30
9	10.466	3.411	0.87	2.98
10	9.611	3.133	0.86	2.70
11	8.827	2.877	0.85	2.44
12	8.106	2.642	0.83	2.21
13	7.444	2.426	0.82	1.99
14	6.837	2.228	0.81	1.80
15	6.278	2.046	0.80	1.63
16	5.766	1.879	0.79	1.48
17	5.295	1.726	0.77	1.34
18	4.863	1.585	0.76	1.21
19	4.466	1.456	0.75	1.09
20	4.101	1.337	0.74	0.99
21	3.767	1.228	0.73	0.89
22	3.459	1.127	0.72	0.81
23	3.177	1.035	0.71	0.73
24	2.917	0.951	0.69	0.66
25	2.679	0.873	0.68	0.60
26	2.461	0.802	0.67	0.54
27	2.260	0.736	0.66	0.49
28	2.075	0.676	0.65	0.44
29	1.906	0.621	0.64	0.40
30	1.750	0.570	0.63	0.36
31	1.607	0.524	0.62	0.32
32	1.476	0.481	0.61	0.29
33	1.356	0.442	0.60	0.26

Year	ΔSOC g C m^{-2}	Emission g CO_2 m^{-2}	Characterization factor[a]	GWP100 g CO_2e m^{-2}
34	1.245	0.406	0.59	0.24
35	1.143	0.373	0.58	0.22
36	1.050	0.342	0.57	0.19
37	0.964	0.314	0.56	0.18
38	0.886	0.289	0.55	0.16
39	0.813	0.265	0.54	0.14
40	0.747	0.243	0.53	0.13
41	0.686	0.224	0.52	0.12
42	0.630	0.205	0.51	0.10
43	0.578	0.189	0.50	0.09
44	0.531	0.173	0.49	0.08
45	0.488	0.159	0.48	0.08
46	0.448	0.146	0.47	0.07
47	0.411	0.134	0.46	0.06
48	0.378	0.123	0.45	0.06
49	0.347	0.113	0.44	0.05
50	0.319	0.104	0.43	0.05
51	0.293	0.095	0.42	0.04
52	0.269	0.088	0.42	0.04
53	0.247	0.080	0.41	0.03
54	0.227	0.074	0.40	0.03
55	0.208	0.068	0.39	0.03
56	0.191	0.062	0.38	0.02
57	0.176	0.057	0.37	0.02
58	0.161	0.053	0.36	0.02
59	0.148	0.048	0.35	0.02
60	0.136	0.044	0.34	0.02
61	0.125	0.041	0.33	0.01
62	0.115	0.037	0.33	0.01
63	0.105	0.034	0.32	0.01
64	0.097	0.032	0.31	0.01
65	0.089	0.029	0.30	0.01
66	0.082	0.027	0.29	0.01
67	0.075	0.024	0.28	0.01
68	0.069	0.022	0.27	0.01
69	0.063	0.021	0.27	0.01
70	0.058	0.019	0.26	0.00
71	0.053	0.017	0.25	0.00
72	0.049	0.016	0.24	0.00
73	0.045	0.015	0.23	0.00
74	0.041	0.013	0.22	0.00
75	0.038	0.012	0.22	0.00
76	0.035	0.011	0.21	0.00
77	0.032	0.010	0.20	0.00

Year	ΔSOC g C m^{-2}	Emission g CO_2 m^{-2}	Characterization factor[a]	GWP100 g CO_2e m^{-2}
78	0.029	0.010	0.19	0.00
79	0.027	0.009	0.18	0.00
80	0.025	0.008	0.17	0.00
81	0.023	0.007	0.17	0.00
82	0.021	0.007	0.16	0.00
83	0.019	0.006	0.15	0.00
84	0.018	0.006	0.14	0.00
85	0.016	0.005	0.13	0.00
86	0.015	0.005	0.13	0.00
87	0.014	0.004	0.12	0.00
88	0.013	0.004	0.11	0.00
89	0.012	0.004	0.10	0.00
90	0.011	0.003	0.09	0.00
91	0.010	0.003	0.09	0.00
92	0.009	0.003	0.08	0.00
93	0.008	0.003	0.07	0.00
94	0.008	0.002	0.06	0.00
95	0.007	0.002	0.05	0.00
96	0.006	0.002	0.05	0.00
97	0.006	0.002	0.04	0.00
98	0.005	0.002	0.03	0.00
99	0.005	0.002	0.02	0.00
100	0.005	0.001	0.02	0.00
Sums		-0.017		-12.86

[a] Based on Petersen et al. (2013)

13 Appendix D: Calculations for Perspectives

This section explains how the total potential GHG savings for US corn production were estimated based on the simplified formula described in Section 4.5. To generalize results to the entire US (and not focus solely on the case studies for Minnesota and North Dakota), the general US corn production process from the ecoinvent 3 database[41] was utilized. The change in GHG emissions per Mg of corn can thereby by estimated as follows.

- $\Delta GWP_{100,\,US} = 0.39\ \text{kg CO}_2\text{e ha}^{-1} / Y_{c,i} + \Delta Y_c / (0.415\ \text{Mg ha}^{-1}) \cdot (-24.1\ \text{kg CO}_2\text{e Mg}^{-1}) - \Delta Y_c / Y_{c,i} \cdot GWP_{100,\,ref.}$ (Equation 13)

where

ΔY_c = 0.19 Mg ha^{-1} (3 bushels per acre, see Perspectives section)
$Y_{c,i}$ = 9.32 Mg ha^{-1} (ecoinvent 2014)
$GWP_{100,\,ref.}$ = 374 kg CO_2e Mg^{-1} (based on ecoinvent 2014, see Section 4.5)

By use of Equation 13, a reduction in GHG emissions of 18.4 kg CO_2e per Mg corn is estimated (4.8% reduction compared to the full carbon footprint of dried corn in the ecoinvent database). With an assumed *P.b.* response rate of 60%, the GHG savings could potentially be obtained for 210 out of the 350 million Mg corn annually produced in the US (rough number based on FAOSTAT 2016). Meanwhile, it may be necessary to apply the *P.b.* inoculant to all US corn fields (including non-responsive fields) to reap the full potential. This will increase the upstream effect and reduce the overall GHG savings. To be conservative, full application (on 100% of the corn fields) is assumed together with the 60% response rate. On this basis, the following potential GHG saving is estimated:

- Upstream effect (based on Equation 2)
  $100\% \cdot 350 \cdot 10^6\ \text{Mg} \cdot 0.39\ \text{kg CO}_2\text{e ha}^{-1} / (9.32\ \text{Mg ha}^{-1}) =$
  $\underline{0.015 \cdot 10^6\ \text{Mg CO}_2\text{e}}$
- Field effect (based on Equation 3)
  $60\% \cdot 350 \cdot 10^6\ \text{Mg} \cdot 0.19\ \text{Mg ha}^{-1} / (0.4\ \text{Mg ha}^{-1}) \cdot (-24.1\ \text{kg CO}_2\text{e Mg}^{-1}) =$
  $\underline{-2.3 \cdot 10^6\ \text{Mg CO}_2\text{e}}$
- Yield effect (based on Equation 4)
  $-\ 60\% \cdot 350 \cdot 10^6\ \text{Mg} \cdot 0.19\ \text{Mg ha}^{-1} / (9.32\ \text{Mg ha}^{-1}) \cdot 374\ \text{CO}_2\text{e Mg}^{-1} =$
  $\underline{-1.6 \cdot 10^6\ \text{Mg CO}_2\text{e}}$
- Total effect (based on Equation 1 and the calculations right above)
  $(0.015 - 2.3 - 1.6) \cdot 10^6\ \text{Mg CO}_2\text{e} =$
  $\underline{\underline{-3.9 \cdot 10^6\ \text{Mg CO}_2\text{e}}}$

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[41] Maize grain {US}| production | Conseq, U (Agricultural/Food/Transformation)

14 Appendix E: Inputs to uncertainty assessment

The present appendix outlines the uncertainty data that was used in the statistical software tool JMP to analize uncertainty of the base case results for global warming.

Corn after corn			**Minnesota**			**North Dakota**			**Both states**		
Symbol in LCA	**Symbol in JMP**	**Unit**	**Mode**	**Absolute CI**		**Mode**	**Absolute CI**		**Relative CI**		**Assumed distribution**
				CI 2.5%	CI 97.5%		CI 2.5%	CI 97.5%	CI 2.5%	CI 97.5%	
$\Delta GWP_{100,\ inoculant,\ area}$	S	kg CO_2e ha^{-1}	3.0	2.7	3.9	3.0	2.7	3.9	N/A	N/A	Triangular
$GWP_{100,\ SOC}$	T_{CO2}	kg CO_2e ha Mg^{-2}	-38.0	-30.4	-45.5	-59.0	-47.2	-70.7	-20%	20%	Normal
$GWP_{100,\ field,\ N2O}$	T_{N2O}	kg CO_2e ha Mg^{-2}	-15.9	-10.0	-21.8	-11.0	-6.9	-15.0	-37%	37%	Normal
$GWP_{100,\ yield,\ N2O}$	U_{N2O}	kg CO_2e ha Mg^{-2}	-13.8	-8.7	-18.9	-16.8	-10.6	-23.0	-37%	37%	Normal
$GWP_{100,\ inputs}$	U_{inputs}	kg CO_2e ha Mg^{-2}	-15.1	-12.1	-18.1	-20.2	-16.2	-24.2	-20%	20%	Normal
ΔY_c	ΔYc:	Mg ha^{-1}	0.44	0.34	0.54	0.37	0.22	0.52	N/A	N/A	Normal
$Y_{c,i}$	Yc,i:	Mg ha^{-1}	10.7	-	-	8.5	-	-	0%	0%	Fixed

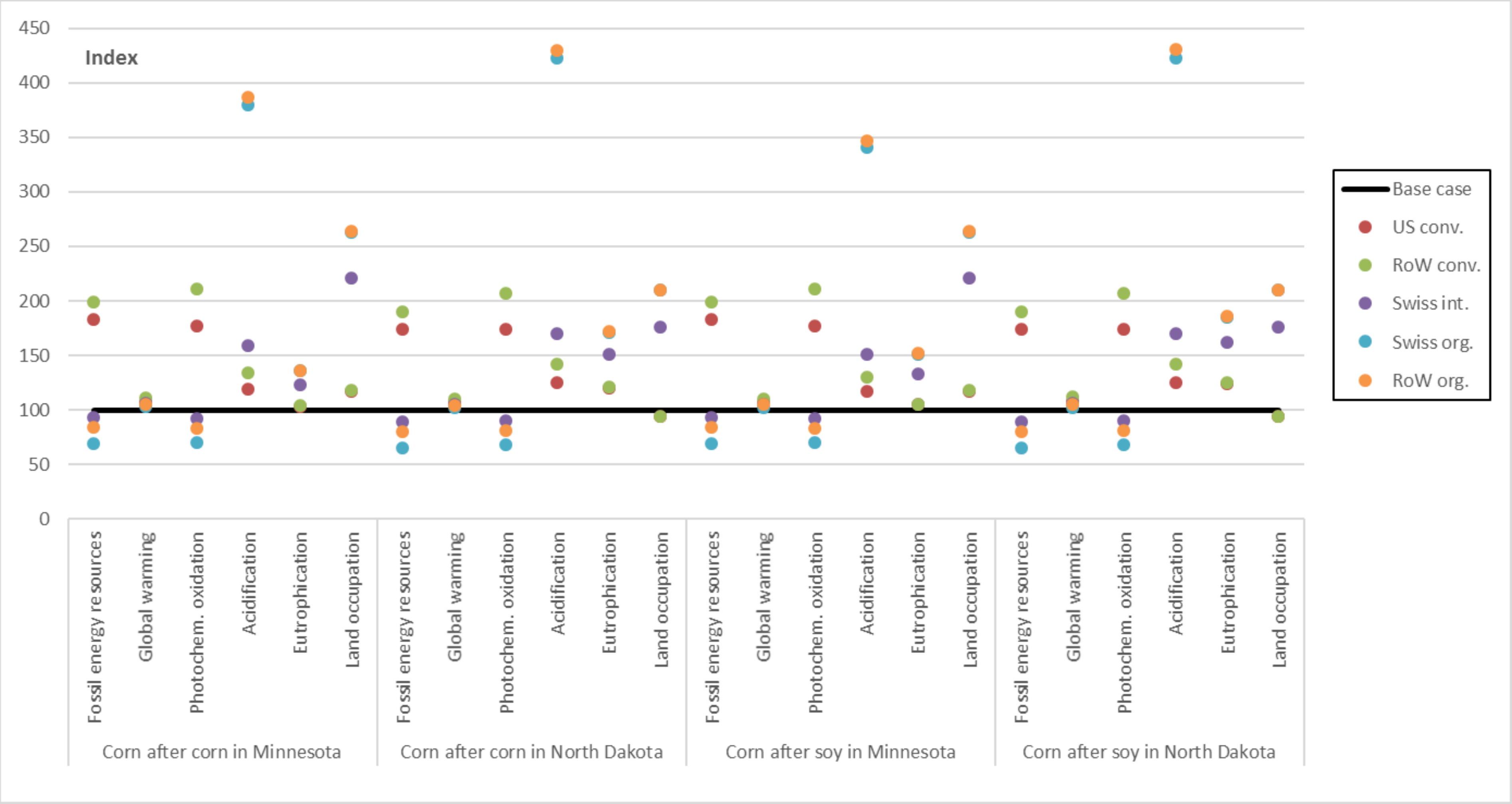
Corn after soybeans			**Minnesota**			**North Dakota**			**Both states**		
Symbol in LCA	**Symbol in JMP**	**Unit**	**Mode**	**Absolute CI**		**Mode**	**Absolute CI**		**Relative CI**		**Assumed distribution**
				CI 2.5%	CI 97.5%		CI 2.5%	CI 97.5%	CI 2.5%	CI 97.5%	
$\Delta GWP_{100,\ inoculant,\ area}$	S	kg CO_2e ha^{-1}	3.0	2.7	3.9	3.0	2.7	3.9	N/A	N/A	Triangular
$GWP_{100,\ SOC}$	T_{CO2}	kg CO_2e ha Mg^{-2}	-49.8	-39.9	-59.8	-49.3	-39.5	-59.2	-20%	20%	Normal
$GWP_{100,\ field,\ N2O}$	T_{N2O}	kg CO_2e ha Mg^{-2}	-10.5	-6.6	-14.4	-5.3	-3.3	-7.2	-37%	37%	Normal
$GWP_{100,\ yield,\ N2O}$	U_{N2O}	kg CO_2e ha Mg^{-2}	-13.8	-8.7	-18.9	-16.8	-10.6	-23.0	-37%	37%	Normal
$GWP_{100,\ inputs}$	U_{inputs}	kg CO_2e ha Mg^{-2}	-15.1	-12.1	-18.1	-20.2	-16.2	-24.2	-20%	20%	Normal
ΔY_c	ΔYc:	Mg ha^{-1}	0.44	0.34	0.54	0.37	0.22	0.52	N/A	N/A	Normal
$Y_{c,i}$	Yc,i:	Mg ha^{-1}	10.7	-	-	8.5	-	-	0%	0%	Fixed

15 Appendix F: Changing assumptions regarding displaced corn

Corn assumed to be displaced →			Base case		US conventional		RoW conventional		Swiss integrated		Swiss organic		RoW organic	
System	**Impact category**	**Unit**	**Yield eff.**	**Total**	**Yield eff.**	**Total**	**Yield eff.**	**Total**	**Yield eff.**	**Total**	**Yield eff.**	**Total**	**Yield eff.**	**Total**
Corn after corn in Minnesota	Fossil energy resources	MJ LHV	-81	-78	-146	-143	-159	-156	-76	-73	-57	-54	-69	-66
	Global warming	kg CO_2e	-13	-36	-15	-39	-17	-40	-15	-38	-14	-37	-14	-38
	Photochem. oxidation	g C_2H_4e	-2	-2	-3	-3	-4	-3	-2	-1	-1	-1	-1	-1
	Acidification	g SO_2e	-117	-88	-134	-106	-148	-119	-169	-141	-364	-336	-370	-342
	Eutrophication	g PO_4^{3-}e	-142	-459	-157	-474	-160	-477	-249	-566	-307	-625	-309	-626
	Land occupation	m^2a	-25	-25	-30	-30	-30	-30	-56	-55	-66	-66	-66	-66
Corn after corn in North Dakota	Fossil energy resources	MJ LHV	-91	-87	-155	-151	-168	-164	-81	-77	-61	-57	-73	-69
	Global warming	kg CO_2e	-14	-39	-16	-42	-18	-43	-16	-41	-14	-40	-15	-41
	Photochem. oxidation	g C_2H_4e	-2	-2	-3	-3	-4	-4	-2	-2	-1	-1	-2	-1
	Acidification	g SO_2e	-122	-82	-142	-102	-156	-116	-179	-139	-386	-346	-392	-352
	Eutrophication	g PO_4^{3-}e	-103	-311	-166	-374	-169	-377	-263	-471	-325	-533	-327	-535
	Land occupation	m^2a	-33	-33	-31	-31	-32	-31	-59	-59	-70	-70	-70	-70
Corn after soy in Minnesota	Fossil energy resources	MJ LHV	-81	-78	-146	-143	-159	-156	-76	-73	-57	-54	-69	-66
	Global warming	kg CO_2e	-13	-39	-15	-42	-17	-43	-15	-41	-14	-40	-14	-41
	Photochem. oxidation	g C_2H_4e	-2	-2	-3	-3	-4	-3	-2	-1	-1	-1	-1	-1
	Acidification	g SO_2e	-117	-103	-134	-120	-148	-133	-169	-155	-364	-350	-370	-356
	Eutrophication	g PO_4^{3-}e	-142	-322	-157	-336	-160	-339	-249	-428	-307	-487	-309	-488
	Land occupation	m^2a	-25	-25	-30	-30	-30	-30	-56	-55	-66	-66	-66	-66
Corn after soy in North Dakota	Fossil energy resources	MJ LHV	-91	-87	-155	-151	-168	-164	-81	-77	-61	-57	-73	-69
	Global warming	kg CO_2e	-14	-33	-16	-36	-18	-37	-16	-35	-14	-34	-15	-35
	Photochem. oxidation	g C_2H_4e	-2	-2	-3	-3	-4	-4	-2	-2	-1	-1	-2	-1
	Acidification	g SO_2e	-122	-82	-142	-102	-156	-116	-179	-139	-386	-345	-392	-352
	Eutrophication	g PO_4^{3-}e	-103	-260	-166	-323	-169	-326	-263	-420	-325	-482	-327	-484
	Land occupation	m^2a	-33	-33	-31	-31	-32	-31	-59	-59	-70	-70	-70	-70

The table above shows the implications of chosing different assumptions about the corn being displaced by additional yield obtained via *Penicillium bilaiae* (impact on yield effect and impact on total change in environmental impacts). RoW stands for ‘Rest of the World’ and the life cycle inventories for the different types of corn stem from the ecoinvent database (cf. Section 6.5).

The graph below shows the total change in environmental impacts in the base case as index 100. The total change in environmental impacts when changing assumptions about displaced corn (see table above) has been indexed in relation to the base case and shown in the figure.



16 Appendix G: ILUC models

It is beyond the scope of the present paper to make an extensive comparison of different ILUC models. Such an analysis can be found in Woltjer et al. (2017). However, a few perspectives are added in this appendix to shed more light on the implications of choosing the model developed by Schmidt et al. (2015). This is done by a comparison to a few other results from the literature relating to US corn.

Since most ILUC results relate to the production of biofuels, a rough ILUC estimate (based on the model by Schmidt et al. 2015) has been derived for bioethanol produced from corn in Minnesota and North Dakota. For this, we use the following data.

• ILUC emissions from corn:	2050 kg CO_2e $(ha\cdot y)^{-1}$	See Section 6.4
• Ethanol yield from corn:	425 l Mg^{-1}	Wang et al. (2012)
• Ethanol energy content:	21.2 MJ l^{-1}	

Based on the corn yield in Minnesota (without inoculant) and the data above, the ILUC emissions from corn ethanol *before accounting for co-produced protein feed* can be calculated as follows:

$$2050 \text{ kg } CO_2e \ (ha\cdot y)^{-1} / (10.7 \text{ Mg } (ha\cdot y)^{-1} \cdot 425 \text{ l } Mg^{-1} \cdot 21.2 \text{ MJ } l^{-1}) = 21.3 \text{ g } CO_2e \text{ MJ}^{-1}$$

The corresponding number for North Dakota is 26.8 g CO_2e MJ^{-1}. These numbers are intermediate steps to the final estimate for an ILUC factor because the effect of co-produced protein feed (so-called distillers grains with solubles or DGS) needs to be factored in. This has not been assessed by detailed modeling in the present study. Instead, it has been assumed that the co-product reduces the 'intermediate' ILUC results by roughly one-third. This is based on Figure 2 (comparison of column 2 and 3) in Hertel et al. (2010). Through this approach, the following rough ILUC estimates are obtained.

- $ILUC_{ethanol, MN}$ ~14 g CO_2e MJ^{-1}
- $ILUC_{ethanol, ND}$ ~18 g CO_2e MJ^{-1}

These results can now be compared to other ILUC results for corn ethanol. A brief (non-exhaustive) overview has been provided below.

Study	Model	Geographical scope	Co-product credit	Time accounting	ILUC fator g CO_2e MJ^{-1}
Searchinger et al. (2008)	GE*	USA	Partly factored in	30 y amortization	104
Hertel et al. (2010)	GE*	USA	Factored in	30 y amortization	27
US EPA (2010)	GE*	USA	Factored in	30 y amortization	26
CARB (2014)	GE*	USA	Factored in	30 y amortization	20
Valin et al. (2014)	GE*	EU	Factored in	20 y amortization	14
Wang et al. (2012)	GE*	USA	Not clear	30 y amortization	9

* General equilibrium

As shown above, the (crudely) estimated ILUC results for Minnesota and North Dakota fall within the lower end of the (non-exhaustive) examples from the literature.

Meanwhile, it is important to keep in mind that the bio-physical ILUC model by Schmidt et al. (2015) differs from the general equilibrium models by not assuming reduced crop consumption but only changes in crop intensity and crop area (see description in Section 6.4). This drives results up (compared to the GE models). On the other hand, the ILUC model by Schmidt et al. (2015) relies on time-dependent GWP[42] for time accounting, which is another approach than the amortization approach applied in the studies listed in the table above. This time accounting approach drives results down (compared to the 20 or 30 year amortization approach). Kløverpris and Mueller (2013) showed that the ILUC factors estimated by Searchinger et al. (2008) and Hertel et al. (2010) would be reduced to respectively 30 and 11 g CO_2e MJ^{-1} if time-dependent GWP (baseline time accounting) were applied. In other words, with consistent time accounting, the (crudely) estimated ILUC results for Minnesota and North Dakota fall somewhere in between those by Searchinger et al. (2008) and Hertel et al. (2010).

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[42] Also referred to as 'baseline time accounting' by Kløverpris and Mueller (2013)

17 Appendix H: Review Statement

Critical Review statement for the study
“Environmental life cycle assessment of US corn produced with microbial phosphate inoculant”
2018

Prepared by an author team lead by Jesper Kløverpris, Novozymes

Review panel:
Prof. Dr. Michael Hauschild, Hauschild Consult, Denmark, Chair and external independent LCA expert
Dr. Lorie Hamelin, Federal University of Toulouse, Institut National des Sciences Appliquées (INSA), Toulouse, France, expert in LCA on agricultural systems and land use impact assessment
Dr. Nuala Fitton, University of Aberdeen, United Kingdom, expert biogeochemical modelling of nitrogen and carbon in soil

References
ISO 14040 (2006): Environmental Management - Life Cycle Assessment - Principles and Framework
ISO 14044 (2006): Environmental Management - Life Cycle Assessment - Requirements and Guidelines

The scope of the critical review
The review panel has the task to assess whether

- the methods used to carry out the LCA are consistent with the international standards ISO 14040 (2006) and ISO 14044 (2006)
- the methods used to carry out the LCA are scientifically and technically valid
- the data used are appropriate and reasonable in relation to the goal of the study
- the interpretations reflect the limitations identified and the goal of the study, and
- the study report is transparent and consistent.

The members of the critical review panel were chosen to ensure the required LCA competence and expertise in the scientific and technical aspects of the studied product system, specifically regarding consequential life cycle assessment of crop cultivation systems and biogeochemical modelling of nutrients and carbon in soil. The review was performed on a final draft of the full LCA report. The analysis of individual datasets and calculations underlying the results are outside the scope of this review.
This review statement is valid for the Final version of the LCA report dated 2018.

The review process
The review was performed on a final draft of the full LCA report and based on ISO 14044 (2006) 6.3 as a critical review by an external panel. The review process took place in February to April 2018 over three rounds. In the first round the panel provided 110 comments of general, technical or editorial nature. The comments

were processed by the author team and a revised LCA report was produced. The panel checked the processing of the review comments and reviewed the introduced changes in the revised LCA report. This second review round resulted in 18 follow-up comments and recommendations. The practitioner processed these additional comments and produced the present final version of the LCA report. The review comments and their processing are documented in the table at the end of the review statement.

General remarks

The study analyzes the environmental impacts on corn production of introducing corn seeds treated with an inoculant containing spores of the soil fungus Penicilium bilaiae (P.b.), which has been shown to mobilize phosphate in the soil and increase its availability for the corn plant. The analysis is performed as a comparative consequential LCA on two types of crop rotation systems (continuous corn and corn/soybean rotation) in two American states, Minnesota and North Dakota, comparing the use of conventional and P.b. inoculated seeds. The scope of the LCA study is found to be appropriate and in accordance with the goal of the study. The data collected for the inventory modelling, and the selection of impact categories included in the study, are found to be adequate, and the handling of the sensitivity and uncertainty analysis satisfactory to substantiate the conclusions regarding the environmental differences between the compared alternatives. Central assumptions (like choice of time horizon for changes in soil organic carbon and assumption of what is affected by the modelled changes in corn yield) are tested in sensitivity analyses to check their influence on the conclusions. Overall the analysis is found to be adequate and the handling of the sensitivity and uncertainty analysis satisfactory to substantiate the conclusions regarding the environmental differences between the compared alternatives. The author team has been very forthcoming in the dialogue with the review panel and ambitious in the processing of the review comments, and important technical improvements to the report were introduced through the review process.

Conclusion

Overall, the critical review found the quality of the chosen methodology and its application in the analysis to be adequate for the purposes of the study and in accordance with the ISO 14040 and ISO 14044 standards. The reporting of the study and its results is transparent. The discussion of the results covers the relevant aspects in accordance with the goal of the study, and the conclusions are well founded on the outcome of the study and in accordance with the defined goal.

On behalf of the review panel

Michael Hauschild

Michael Z. Hauschild
Chair of review panel
27 May 2018

Template for Critical Review comments

Date: 2 May 2018	Document: Environmental life cycle assessment of US corn produced with microbial phosphate inoculant

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No.	**Rev. ID**	**Clause No./ Sub clause No./ Annex** (e.g. 3.1)	**Paragraph/ Figure/Table/ Note** (e.g. Table 1)	**Type of commint[2]**	**Comment (justification for change)**	**Proposed change**	**Decisions** on each comment submitted
1	MH	Summary	Para 2	ed	“The purpose of this report is to perform an environmental life cycle assessment (LCA)…”	the purpose of the report is to *document* an environmental life cycle assessment, performed in a study …	The sentence has been changed so it now begins: ‘The purpose of this environmental life cycle assessment (LCA) is to…’
2	LH	Summary	Para 5	te/ge	This is the only place where it is mentioned that marginal data are used. Yet, how the authors strive to do that is not clear in the report. For example, for N fertilizers, a mix based on the ratio used in Ecoinvent was used (section 3.2). Is it, at least, based on the consequential dataset of Ecoinvent?	Specify, where appropriate, how it is ensured that marginal data are used.	All inputs to corn production (including the mix of N fertilizers) are based on the consequential dataset of ecoinvent. This has now been specified in Section 3.2
3	MH	Summary	Para 7	ed	“The introduction of the P.b. inoculant is estimated to reduce the impact of corn production…” “estimated” indicates that you expect this but your study tells you that this is the case	Replace “estimated” by “found” or similar	Proposed change adopted
4	LH	Summary	-	te	One could suppose that since the inoculant releases organic acid allowing to break phosphate bonds, the soil pH will decrease. This could mean more lime to be applied in the P.b. system. Lime was excluded from the inventory for its negligible impacts, but I think this should be part of the discussions (i.e. if a more acidic soil is expected with P.b. in the long-term, and consequences this could have)	Discuss the potential implications on soil pH	Discussion added in Section 3.2 (third paragraph)
5	LH	Summary	-	te	The principle of the inoculant seems to be based on the fixed P pool. To fully capture the inoculant effects in the short-to-longer term, it would be important to present an understanding of the P dynamics as well. For instance, one could ask for how long there is enough soil P (fixed pool) for obtaining the increased yields found here. Follow-up comment: This sub-section is a great addition, but my above main concern is not addressed. To be more specific: To obtain the measured yield increases, one pre-condition is the availability of mineral phosphate in the soil (/root zone). As rotations are grown on a given land, and as more P ends as uptake by the plant, this “mineral phosphate	Discuss, where relevant, if concerns on long-term P availability (from fixed pool) are relevant, vs achieving the yields presented herein. Could you comment on that? Can it be that after some years, the initial yield increases are no longer achieved, because of the lower P stock? Or is this an unlikely hypothesis and if so why?	In Section 4.6 of the revised report, a sub-section has been added, which covers phosphorus resources and phosphorus dynamics – including considerations of long term effects. Decision on follow-up comment: A new sub section (3.1.2) discussing long term yield increases has been added under Section 3.1.

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2 **Type of comment:** **ge** = general **te** = technical **ed** = editorial

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					stock" in the soil will likely decrease through the years.		
6	MH	Introduction	Para 1	te	The inoculant treatment might also hold potential to save use of P-fertilizer and hence the use of non-renewable resource phosphate rock for P-fertilizer production, yet this is not mentioned anywhere in the report?	Comment on whether this is a perspective in the technology	Comment added at the end of Section 4.6.1
7	MH	Introduction	Para 1	te	"…the LCA methodology (ISO, 2006a; ISO, 2006b) allows for inclusion of a broader range of resource and impact categories." ISO 14044 actually not only allows but *calls* for a broader range of categories. – ISO 14044 (4.4.2.2.1) states that "The selection of impact categories shall reflect a comprehensive set of environmental issues related to the product system being studied, taking the goal and scope into consideration. "	Clarify this in the text	This has now been clarified in a footnote related to the sentence pointed out.
8	LH	2.1.4	Para 1	te	The study performs several comparisons, e.g. using the surplus yield to relieve the pressure on land demand (i.e. reduce the overall demand for corn), or for bioethanol production. There is no comparative assertion ("environmental claim regarding the superiority or equivalence of one product versus a competing product that performs the same function"), but since the comparisons are part of the conclusion this would be useful to clarify.	Adjust the statement to reflect that some comparisons are made and conclusions are drawn from it.	It has now been specified (in Section 2.1.4) that such comparisons are made.
9	LH	2.1.6	Para 1	te/ge	At this stage, it is not clear what a responsive corn field is (responsive to what). It becomes clearer in the conclusion only (p.43). Follow-up comment: OK, but it would then be necessary to mention, early in the report (e.g. section 3.1), that the yield increases considered herein apply to RESPONSIVE corn fields (in Minnesota and North Dakota). And that these responsive fields are estimated to represent ca. 60% of the US fields.	Add a brief explanation for "responsive corn field" Please clarify in text	After considering this comment, it has been decided to remove the mentioning of 'responsive fields' under Limitation. The reason is that the study actually does consider responsiveness as part of the perspectives. Decision on follow-up comment: This topic has been revisited and discussed with the lead author of the yield increase

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							paper (Leggett et al. 2015). The average yield increase data for Minnesota and North Dakota (Table 2) actually builds on all available field trials, incl. those with no detected increase in yield. This has been clarified in the text in Section 3.1. In addition, the statement about responsiveness under *Impact Assessment* in the Summary has been modified so it no longer states that results apply to responsive fields (but simply to corn fields in Minnesota North Dakota). It has also been checked whether the changes mentioned above result in any inconsistencies in the remainder of the report. That is not the case. The discussion of responsive corn fields across the US (the 60%) has been maintained in Section 8.2 (Perspectives).
10	LH/ MH	2.2.2	Para 1	te/ge	Why North Dakota and Minnesota? If to represent the whole of US corn, as mentioned here and in the goal definition (P.10), it seems little representative to choose two adjacent states. And already, there are differences between two seemingly similar “Northern Prairies” production systems, so representing the whole of US corn	Add a brief clarification on why it is representative to select these two states to draw the final conclusions that are made in the study.	The section now discusses corn production in Minnesota and North Dakota. The LCA report does not claim that the two states are representative for corn production in the US

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					with these two states appears a little weak, as it stands now. Also, Leggett et al. (2015) did make tests in other states (e.g. why was Nebraska not selected, where the lowest yield deviation – 0.037 Mg ha^{-1} - was obtained?)		as a whole – but a justification of the extrapolation to the domestic level has now been added. The revised report explicitly specifies that it is a crude extrapolation, which has been conducted (as did the draft report).
11	LH	2.2.3	Para 1	ge/ed	One key reference is the work of “Leggett et al. 2014”. In Scopus (and on the paper itself), however, this paper is listed as 2015.	Adjust 2014 for 2015	The author team had been using the PDF published online in 2014. The publication year for the printed version (2015) has now been used in the revised report.
12	LH	-	-	ge	I miss a brief description of the principle behind the inoculant, i.e. how it works. This would help the reader understanding/judging the LCA better.	Add a brief description of how the inoculant allows a greater yield (all mechanisms involved, as in e.g. Leggett et al. 2015, p.1465 at bottom)	A brief description has now been added to the last half of the introduction.
13+ 14	LH/ MH	2.2.5	Para 1	te	Handling of cut-offs not clear. How is the “low importance” assessed? E.g. at page 20, you mention <0.25% for excluding lime. Was this the cut-off consistently applied?	Describe the quantitative / qualitative procedure used to establish the cut-off You should define what low importance means. What is the maximum change caused by the omissions that you have made in the study? A cut-off criterion of 0.25% of environmental impacts for all categories mentioned for omission of liming (p. 20)	We have now quantified the maximum accepted impact from omissions made in the study and mentioned how omissions have been assessed on a case-by-case basis in relation to the quantitative cut-off criteria.
15	LH	2.2.6 / general	Page 12, last para	te	It is stated that if the yield is increased, the least competitive production will likely be displaced. Though I do agree with this rationale, it is not what was applied in the study. The study rather assumes the displaced corn	Clearly mention what inventory is used for the displaced yield (see comment 64). Also discuss the inconsistency with the consequential LCA rationale (partly done in page 40) and how results	We have added a footnote in relation to the term ‘less competitive’, which clearly mentions that it is ‘standard

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					to be identical to the reference corn studied herein. Yet, that "least competitive" corn may come from a place where the inventory is significantly different (inputs, etc.).	could have been affected (at minima) if another inventory would have been used. At best, add a sensitivity analysis. Would it be more likely to be an inventory with higher fertilizer inputs, or lower?	corn production' without the use of *P. bilaiae* (i.e. 'reference corn') – corresponding to typical system expansion in other agricultural LCAs. The footnote goes on to discuss what is actually the 'less competitive' crop production ultimately affected and links this to the ILUC analysis, which is later incorporated in the study. The point made is that it may not be corn, which is ultimately affected. For the same reason, we don't see a need to conduct an analysis for displaced corn with a different inventory. One can either conduct a 'standard system expansion analysis' (as in our base case) or a full-blown ILUC analysis (as in our sensitivity analysis). Applying a modified inventory for 'less competitive' corn would, in our view, fall between two stools.
					Follow-up comment: The footnote is a good addition. Indeed, "a less" (or "the least", as one would typically expect in consequential LCA) competitive supplier(s) is(are) taken off production, and this may not be maize. It also depends of the market(s) considered for that maize stemming from extra yield (animal feed, flour/staple food, ethanol, etc..). For example, in Tonini et al. (2016) (doi: 10.1111/gcbb.12290), we documented, based on FAO historical data & FAPRI outlook towards 2022, that the most competitive marginal carbohydrate/energy-feed (for	An analysis of the sensitivity of yield effect on choice of system expansion is not necessary if at least this (i.e. having the "yield effect" to its lowest possible value) is discussed semi-quantitatively, in terms of the implications it could have on the results – along with an acknowledgement that the current proxy used is not the most conservative one.	Decision on follow-up comment: A change in N fertilizer (as discussed by the reviewer in the follow-up comment) would also mean a change in yield, change in nutrient run-off, change in N_2O emissions, etc. In other words; a whole new corn inventory. Instead of trying to

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					the animal feed market) is likely to be maize. The least competitive one was identified as the one with the lowest annual increase in 2012-2022, being sorghum (but Tonini et al. did not document from which location the "least competitive" sorghum is likely to stem as the interest there was to identify the most competitive energy feed). So yes, it may not be maize that is displaced, it may not be a single crop either but rather a mix of crops, but just because it may not be maize does not justify in itself not going further (one could go further with displacing that other crop/or crop mix). Here, when the authors apply their 'standard system expansion analysis' (one alternative approach applied, as highlighted by the authors, is a full system expansion also including the avoided iLUC; sensitivity section) they considered 'standard corn production' without the use of P. bilaiae to be "avoided" by the increased yield, and this avoided corn is labelled as "yield effect". The breakdown of this effect is presented in Tables 8/9. So 'standard corn production' without the use of P. bilaiae is used as a rough proxy to represent this "yield effect". The point raised by this original comment, thus, was: To which extent the yield effect matters to the final results? What if it (the yield effect) was reduced by e.g. half? This would lead to results even more "extreme", or conservative, than those presented currently with this "standard system expansion analysis" because the difference between the US maize inoculated with P.b. and the one not inoculated would be reduced (as the negative "yield effect" would become smaller). Tables 8/9 show that N_2O emissions and those related to producing the N fertilizer account for more than 60% of the yield effect. If an inventory with e.g. less fertilizers is considered (e.g. a case where "a less" competitive supplier would be one using much less fertilizers), how would this reduce the yield effect?		build a new consistent inventory (to explore the sensitivity to assumptions regarding displaced corn), it was chosen to select five existing inventories from the ecoinvent database with different characteristics in terms of fertilizer and other aspects (conventional, integrated, and organic). This analysis has now been described in Section 6.5 and (the new) Appendix F.
16	MH	2.2.6.1	Para 1	te	Meaning of the index "i" is not clear from neither text nor figure. You may also want to explain the indices "c" and "s" as well although they become clear as you read into	Please explain the meaning of the indices	The indices have now been explained in Section 2.2.6.1 (right before Fig. 1 appears).

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					the description of Figure 2.		
17	NF	2.2.6.1 and 2.2.6.2	Fig 1 & 2	ge	No reference is made about the length of the experimental field trial, are they 2 year or long term trials	Add in reference to length of field trial	Fig. 1 and Fig. 2 represent the conceptual modeling approach applied in the LCA. They do not seek to describe the field trials upon which the LCA rests. This has now been specified in a footnote relating to Fig. 1 as well as in a footnote relating to Fig. 2.
18	NF	2.2.6.2 pg. 16	Para 1	ge	"emissions coming from the field will not be the same…" statement of what the results will be should probably not be made in a methodology section	Please remove statement Follow-up recommendation: Please add reference then to confirm statement	We agree that results do belong in a methodology section. Meanwhile, the statement (which is based on theoretical knowledge) is intended to explain the use of biogeochemical modelling as an integrated part of the LCA. Hence, it has been decided to leave the statement as it is. Decision on follow-up comment: We had considered the statement common knowledge but have now modified the it so it no longer 'predicts' results but only states that *'emissions coming from the field (throughout the full crop rotation) will not necessarily be the same in the two systems'*. In addition, we go on to state that *'The potential difference in field emissions can be estimated through biogeochemical modeling…'*. We believe that

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							these theoretical statements can be made without a reference.
19	NF	2.2.6.3	Para 3	ge	Why was a 40 year period selected	Include reference or statement as to why 40 years is chosen	Included further explanation of the 40-year time period in report along with references.
20	NF	2.2.6.3	Para 3	te	How was default value of 0.2 selected, model recommendations or previous calibration work at site	Please include more detail about this parameter	Further description provided on parameter calibration
21	NF	2.2.6.3	Para 3	te	Was the change in P fraction site specific or a generic change that could be equally applied across all sites	Please include more detail about the change to this parameter	Further description provided on parameter calibration to explain site specific calibration
22	NF	2.2.6.3	Pg 17 para 1	ge	Please add in more detail about soil C spin up and validation as this is an important aspect of the study	Additional section could probably added in the appendix	A generalized description of the soil C spinup methodology was added to the report.
23	MH	2.2.6.3	Pg 17 para 1	te	You mention that "In the long term, changes in SOC will be insignificant whereas they can be substantial in the short term."	Please explain why	Explanation added as a footnote to the same sentence.
24	NF	2.2.6.3	Pg 17 para 2	te	How was SOC converted into CO_2 emissions? Please include equation, reference. How does this compare with other ways of doing it with DayCent?	More detail on the approach is needed here Follow-up recommendation: Last sentence in footnote should be supported by reference	The conversion from SOC to CO2 was based on stoichiometry, which is consistent with DayCent modeling. This has now been specified in a footnote. Decision on follow-up comment: The last sentence in the footnote has been broadned out to cover biogeochemical modeling in general and a refernce has been added.
25	MH	2.2.7	Table 1	te	"Nutrient enrichment (eutrophication)" is called "eutrophication" throughout the rest of the report	Use one of the two consistently	Eutrophication is now used as the 'main term' for this

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							impact category (and 'nutrient enrichment' is mentioned in parentheses in the summary and in Table 1)
26	MH	2.2.7	Table 1	te	Photochemical ozone formation: I believe that NOx also has a characterization factor in the CML method? The impact also has effects on natural vegetation.	Check and correct	There is no generic characterization factor for NOx in the CML method (but there is for nitric oxide and nitrogen dioxide). It has now been mentioned (in Table 1) that the impact also has effects on natural vegetation.
27	MH	2.2.7	Table 1	te	Energy resources: If this impact category only covers fossil energy resources, it should be renamed to reflect that	Check and correct (throughout the report)	It has now been specified (throughout the report) that the impact category covers fossil fuels.
28	MH	2.2.7	Table 1	te	Agricultural land use: If only land occupation is covered under this impact category, I suggest that you consider calling it that – "Land occupation"	Consider and revise as appropriate	Proposal adopted – and, in addition, a clarifying note has been added to Table 1, specifying the land uses included in the category.
29	LH	2.2.7	Last para	te	Why did you select the CML IA baseline LCIA method? Did you consider updates to it (e.g. for global warming, in accordance to Assessment Report 5 of the IPCC)? Also, are there any reasons to believe that results would look different with another method? Follow-up comment: The statement is very strong considering the range of available methods and the vast differences between their characterization factors for some of the impact categories (not least water use, land use and toxicity-related categories). For the limited selection of categories that you consider in this study, there is less variation, and it is probably a correct statement, but this should be specified	Specify why this method and if any emissions factors were updated at the light of best available data. Rephrase as: "For the chosen selection of impact categories, there is no reason to believe that the choice of other impact assessment methods would change the conclusions of the present study" Regarding Table 1: Please add ‡ where it belongs (after land occupation) in the table	We have now added a discussion on the choice of method beneath Table 1. Decision on follow-up comment: Both proposed changes adopted.
30	MH	3	Para 3	ed	Reference to Fig. 2 should be to Fig. 3?	Check and revise	Reference revised

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31	NF	3.1	Para 1	ge	If there is no statistically significant difference between the yields (with and without inoculant) in North Dakota then why include it in the study? If the reason as to why in N. Dakota there is no difference, i.e. site characteristics, climate, has this been lost by the soil and climate selection here	More detail on the approach is needed here	There *is* a statistical significant difference between the yields (with and without inoculant), also in North Dakota. The intension with the text was to explain that no statistical difference for the corn yield increase could be shown based on previous crop. The text has now been simplified and (hopefully) sharpened to make this point clear.
32	MH	3.1	Para 1	ge	Reasoning not clear? Statistical significant difference between with and without P.b. for both continuous corn and corn-after-soybean in Minnesota, but no significant difference between yield increases obtained with P.b. for the two rotation systems? On this basis averaging yield increases across the two systems? And using the Minnesota data to represent North Dakota as well? Why – what were the yield results for North Dakota?	Please comment and revise as appropriate	The text has been revised (see decision in relation to previous comment). Minnesota data was not used to represent North Dakota (Minnesota data was only used to investigate if a significant difference in yield increase could be detected based on previous crop).
33	NF	3.1	Para 1	ge	The Leggett paper was published in 2015 not 2014	Please check reference	Year corrected (cf. response to comment 11)
34	NF	3.1	Para 1	ge	Leggett et al describe an effect due to the plot size, how did the modelling approach address this. Is it possible to add in figure or table showing modelled v's measured yields	More detailed statement on how field trial data was handled addressed (or if addressed, reference where in document it has been)	As stated in the first paragraph of Section 3.1, the LCA focuses on yield observations from large plot field trials. The effect due to the plot size ('the edge effect') has now been described in the last paragraph of Section 3.1. The LCA approach incorporates the edge effect through the above-mentioned

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							use of the average (state-specific) yield increase data from the large plot field trials. This should now appear from the added discussion of the edge effect.
35	MH	3.1	Para 2	te	Why does N-application depend on previous crop if K (also very water-soluble) doesn't and why is P (which mainly remains in the soil) independent of previous crop?	Please comment on this	Soybeans fix nitrogen from the air and some of this remains available in the soil for next year's crop, thereby reducing the need for N fertilizer. This has now been specified in a footnote. Soybeans do not influence K and P fertilizer recommendations for next year's crop.
36	NF	3.1	Table 2	te	Leggett et al states that P fertiliser history could have had an influence on yield differences between the different plot sizes, does this affect P estimates reported in table 2	Please comment on this	A paragraph has now been added at the end of Section 3.1, which discusses P fertilizer history as well as the 'edge effect' and specifies that these have not affected the P estimates reported in Table 2 (which are in line with general recommendations as outlined in Appendix A).
37	NF	3.1	Table 2	ed	"Deviation" do you mean difference in long term average yields	Clarify sentence	Table 2 does not refer to deviation. However, the subsequent Section 3.1.1 does. Here, it has now been clarified (in a footnote) that "deviation" is the mean deviation between the yield of corn with and without the *P.b.* inoculant (all based on large plot field trials

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							documented by Leggett et al. 2015).
38	LH	3.1	Table 2	te	Some inconsistencies in this table for total N, “corn after soybean”. Looking at Appendix B, the value for Minnesota is [fertilization + N fixation] (and it actually gives 156, not 158), while for North Dakota, the value given here is just [N fixation].	Please check that the N fertilizer input used for the inventory is correct (i.e. as stated in Appendix B, or check that Appendix B is correct).	The data on N fertilizers for corn after soybeans in Table 2 is not directly comparable to the average annual input of N fertilizers to the corn-soybean rotations in Appendix B. This has now been specified/explained in Appendix B.
					Follow-up comment: Thanks – I realize my comment should have been clearer, and your answer actually clarifies a wrong assumption from my side regarding the values in Table 2. In fact, Appendix B is very clear on the breakdown of the N input (N from fertilizers, from symbiotic N fixation, from deposition). What is not clear is actually Table 2, for the line “Total N, corn after soybean”. Based on the table title “yield and fertilizer data applied…”, I understand that this line represents the purchased mineral N fertilizer being added (or N from fertilizers of Appendix B). Is it correct? And if so, should the corn-soy value not be 84 and 56.5 kg N ha-1 (Minnesota and N. Dakota, respectively)? And if, as mentioned above, Table 2 and Appendix B are not directly comparable for corn-soy (but it matches perfectly for corn-corn) in terms of “N fertilizer input”, why is it so? Why would “average values” and “Daycent values” match for corn-corn, but not for corn-soy?	Please comment and adjust text as relevant	Decisions on follow-up comment: It has now been specified in a note in Table 2 that ‘Total N’ means ‘purchased mineral N fertilizer added to corn production’ - in accordance with the reviewers understanding. It has now been specified in a table note in Appendix B that data on N fertilizers cover one full crop rotation (i.e. the corn-soybean rotations contain N fertilizer for corn *and* soybeans). The data in Table 2 and Appendix B are consistent and correct. It matches perfectly for corn after corn because the ‘rotation’ is only one year long and N fertilizer is added to corn.

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							The reason that Table 2 and Appendix B are not directly comparable is that Table 2 only contains N fertilizer for corn whereas Appendix B contains N fertilizer for corn as well as for soybeans. This is also why "average values" and "Daycent values" match for corn-corn, but not for corn-soy. Please, also see text added below the table in Appendix B the first revision of the LCA report.
39	MH	3.1	Table 2	ed	Not clear what note "‡ Standard error (Leggett et al. (2014)" refers to?	Check and delete if not used in the table	Now deleted
40	LH	3.1	Table 2 / Appendix A	te	How is the non-negligible difference in macronutrient inputs (N, P, K) between two adjacent states explained?	Add a brief explanation to clarify (is it due to legislation, etc.)	A brief explanation has been added as the second paragraph following Table 2.
41	LH	3.1.1	Para 2	te	I see these deviation values in Table 5 of Leggett, but I do not understand how it is mathematically possible that delta mean yield of two series (with and without P.b.) is not equal to the mean of all individual deviations of the series. I.e. (a+b+c)/3 - (a1+b1+c1)/3 = (1/3) * [(a-a1)+(b-b1)+(c-c1)]. Of course, this equality is not true when we consider absolute values for the parenthesis (i.e. if one series is not consistently higher that its counterpart). But here, we consider yield increases (absolute deviation that are yield decreases should not be considered). Hence, I would have expected the equality to be true. Maybe some "statistical correction" was applied and these deviations of Leggett are not calculated as supposed above. If so, it would help to specify it. Else, it would be more sound to use the difference of the average yield throughout (as it was also used for DayCent, anyway).	Specify what exactly these "deviations" are, in case it involves some statistical correction. Specify also from this point very clearly that these deviations are used rather than the differences in mean yield from Table 2 (else it can take a while to realize it).	The deviation expresses the statistical change in yield obtained with the *P.b.* inoculant. This has now been specified in Section 3.1.1. Besides, the info on 'standard error' has been deleted from the report because it is not used in the LCA (was only included for information). It has further been specified that it is the yield deviations in Section 3.1.1 that has been used for the modelling – and the calibration of yields in DayCent has been discussed

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							(incl. a small discrepancy in yield deviations for corn after soybeans). Finally, the third recommendation of the report (Section 8.3) has been modified as it was realized that the DayCent modelling team had actually modeled the correct yield deviations from the start. The third recommendation of the draft LCA relied on a misunderstanding (which has now been cleared).
42	LH	3.2	Table 3	te	P input is the same between the reference and P.b. systems. I would have expect the technology to involve lower P input (see comments 21, 43-44). Why is this not the case?	Clarify, where relevant, why the same amount of P input are needed whether or not P.b. is applied.	Comment added at the end of Section 4.6.1
43	NF	3.2	Table 3	ge	Are there any other management events such as cultivation, harvesting etc. at the sites?	Add in	Clarifying note added at the bottom of Table 3
44	NF	3.2	Para 2	ge	Reference for assumption as to why all P fertiliser is diammonium phosphate (i.e. Fertiliser handbook stats) and is it relevant for modelling	Add reference	The assumption is based on the ecoinvent dataset. The text has been modified to make this clearer. The exact type of P fertilizer has no relevance for the DayCent modeling and only very minor influence on the LCA results (upstream production of the fertilizer).
45	MH	3.4	Table 4	ed	Fossil energy category is called Energy resources in Table 1	Please use consistent names for the impact categories throughout the report	The name for this impact category has now been updated throughout the report (cf. decision related to comment 27)
46	LH	4	-	te	Would the inoculant also affect the non-harvested (e.g. stover) / non-harvestable (e.g. below-ground biomass	Clarify the partitioning of the increased yield (above and below ground, and for the above,	A clarifying footnote has now been added to the paragraph

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					with e.g. deeper roots) yield, and thus the input of C and N (among others) to the soil? If so, is this considered in the DayCent calculations? It is partly clarified in the sensitivity analysis that no increases in below-ground biomass is considered in the baseline, but it would help to have it clearly here.	between harvested yield and "residues") and ideally how/if it is considered in DayCent.	right beneath Table 5 (in Section 4.1).
47	LH	4.1 & 4.2	Table 5 + 6 + 8 + 9	ed/te	For crop produced: not clear, if it is Mg DM, Mg fresh weight, or Mg dried grain (although one can guess it is not the latter in Table 6, involving soybeans).	Specify the unit Mg (i.e. Mg of what), wherever possible, to avoid any possible confusion. From checking the field CO_2 of continuous corn for Minnesota, it seems to be the same "type" of Mg for all 4 tables (Mg dried grains).	It has now been specified (in Tables 2, 5, 6, 8, and 9) that the corn yields listed are in equivalents of dried corn. Specification also added for soybeans in Table 6.
48	LH	4.1	Table 5	te	A brief explanation for all emissions would help (e.g. are NH_3 emissions just from decaying crop residues, or also from volatilization as fertilizers are applied, etc.). Also for CH_4, is it the reduction in CH_4 uptake by the soil induced by N fertilizers? For N_2O, does it also include the "indirect" emissions from the re-deposition of N, for example?	Explain what these emissions are due to.	Enhanced description of emission losses related to Table 5 from DayCent were added to the report.
49	LH	4.1	Table 5 / text below	te	I understand the principle of the inoculant to be based on more P availability from the soil (see comments 8, 43-44). But the text here explains that there is also an increase in plant N uptake. A few more explanations on why this is the case would help.	Explain why/how N uptake is enhanced with P.b.	Explanation added in a clarifying footnote.
50	LH	4.1	Table 5	te	Linking to the above comment: the P losses are not changed from the reference to the P.b. system. This involves that inoculated crops manage to use all P released. How sure are we about this? Does it stems from a result, or an input to DayCent? Or would there not rather be an improvement in P losses, as observed for N?	Discuss why P losses are the same between the reference and P.b., for a given state. In other words why P losses are not affected by the introduction of the inoculant technology?	Some reasoning for this effect has now been discussed in the report.
51	NF	4.1	Table 5	ge	It is interesting that in the cropping system there is an accumulation of SOC. Is the site cultivated every year? Without information on the soil C spin up etc. it is difficult to know if this is a real effect or a modelling one	Discussion as to why soil C accumulates in a cropping system	Information on the soil C spin up has been added in Section 2.2.6.3 and a discussion of historical practices has been added in the first paragraph after Table 5. As mentioned in the text, assumptions of the

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							cropping history can influence the absolute rate of C change. Hence, there is indeed some uncertainty related with the gains of SOC (i.e. the negative CO_2 emissions) listed in Table 5. Meanwhile, biogeochemical models (such as DayCent) are intended to be used for determining relative changes between systems and not as well conceived for determining absolute carbon stock levels (even though it is understood that the absolute stock levels could have some impact on the relative differences). Ideally, soil carbon stock changes would be measured to allow for more precise calibration of the management (tillage, C inputs) on initial SOC levels. Meanwhile, results between treatments should still be defendable and DayCent's strength in modeling relative changes matches well with consequential LCA that also focuses on the change from one state to another.
					Follow-up comment: The amended text is great.	It would be nice to see a numeric value of the modelled soil C added to the text or tables. This will help understand how degraded the soil C is.	Decision on follow-up comment: It has now been specified in Section 2.2.6.3 that initial soil carbon was 57.4 Mg ha^{-1}.

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52	MH	4.1	Table 5	ed	What is the meaning of the dot after NO (nitric oxide)?	Consider and delete if appropriate	NO includes an unpaired electron (free radical), which is denoted by a dot in the chemical formula. No changes made in report.
53	MH	4.1	Table 5	ge	Negative CO_2 emissions for all schemes indicating that SOC is increasing regardless of continuous corn cultivation practice. This contrasts with Danish findings that SOC is depleted by current agricultural practices but this is perhaps not the case in these locations?	Please comment on this	Some insight into this behaviour is now reported in this section of the report. (See comment # 51)
54	NF	4.1	Table 5 and 6	ge	It would be nice to see the emissions from the different GHG's converted into CO_2 equivs so the reader can directly understand the differences between the sites and presence of inoculant	Add in row with CO_2 equivs	Table 5 and 6 covers emissions contributing to a number of different impact categories. It would mess up the table if these contributions should be characterized in terms of the indicators listed in Table 1. Hence, the table remains unchanged. However, the request from the reviewer is partly covered by the overviews in Table 8 and 9 where emissions have been presented in terms of CO_2 equivalents.
55	NF	4.1	Entire section	ed	"emissions to air" should probably be to atmosphere	Change accordingly	Probably, yes. Meanwhile, 'emissions to air' is 'ecoinvent nomenclature' and has been kept as is.
56	NF	4.1	Table 5 and 6	te	If soil C is specifically calculated to account for the change in "phases" i.e. accumulating C to equilibrium by averaging GHG emissions do the authors assume that this phase change will not affect the rate of GHG emissions?	Clarify, add statement to part of document where this is discussed and impact (if any) or add in values for confirmation	A clarifying statement has now been added beneath Table 7.
57	NF	4.1	Table 5 and	ge	Are the emission units for nitrous oxide correct? The	More detail as to how modelled estimates fall	The N_2O emissions reported

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			6		nitrous oxide emissions seem to be a bit on the high side (e.g. IPCC emission factor = 1% whereas here it seems to be more like 3%). Does any reference exist about similar rates from corn/soybean production systems in N. America	within similar systems	in Table 5 are very much consistent with values reported in the region for corn cropping systems. Wagner-Riddle (1997 & 2007) reported annual emissions of ~3.85-4.1 kg N_2O ha^{-1} yr^{-1} for corn systems in southern Ontario. In Minnesota, Johnson et al., (2010) reported emissions over 3 years of 4.97-5.57 kg N_2O ha^{-1} yr^{-1} across treatments with soybean years averaging 3.89-4.41 kg N_2O ha^{-1} yr^{-1}. Supporting references for Table 5 results from regional studies have been added to report.
58	NF	4.1	Table 5 and 6	ge	Are values here on a per year basis or over the growing season	Amend units here and throughout report to be ha^{-1} yr^{-1} or growing season	The caption above Table 5 specifies that the listed emissions are annual emissions. The caption above Table 6 specifies that the listed emissions are per 'one corn-soybean rotation (two years)'. This is considered adequate and hence no change has been made.
59	NF	4.1	Table 6	ge	Are the GHG emissions calculated over the full two year rotation or single, is it possible to separate them out as done with yields	Clarify	It has been further clarified in the text that the GHG emissions have been calculated over the full two-year rotation. It would be possible to separate out the emissions but it would not be relevant for the LCA as

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							discussed in Section 4.3.
60	LH	4.1	Table 7	ed/te	In the table title, the unit mentioned is incorrect; one should read Mg CO_2 ha^{-1} y^{-1} (rather than just Mg CO_2 $ha^{-1)}$ as the presented figures are already annualized.	Correct the table title Mg CO_2 ha^{-1} y^{-1} and make it clear that all figures presented in the table are annualized.	The caption specifies that results are average emissions per '*one crop cycle (one year for continuous corn and two years for corn-soy rotations)*' and the table specifies the different time perspectives (over which the emissions have been averaged). Since results are shown per crop cycle (which is two years for the corn-soybean rotation), it would be incorrect to use the unit 'Mg CO_2 ha^{-1} y^{-1}'. Hence, the table remains unchanged.
61	MH	4.1	Last para	ge	Why should it be expected that "the difference between the reference systems and the corresponding P.b. systems generally become smaller with time" as you state?	Please provide a reason for this expectation	A reason has now been provided with reference to previous discussion in the report of SOC equilibrium levels.
62	LH	4.2	Tables 8/9	te	Drying and transport are inconsistent. The aim here is to reflect the additional amount of grains that undergo transport and drying in the P.b. inventory. In "yield effect", the impact of avoiding to produce & dry & transport 1 Mg maize is shown. For Minnesota, the additional drying and transport are included in the P.b. inventory, and the "yield effect" includes the avoided drying but not transport. For North Dakota, the additional maize for transport is not included (while it is for drying). And the "yield effect" is here including both transport & drying.	Correct the inconsistency.	The previous column heading 'Yield effect' was misleading because the column includes transport and drying of displaced corn (which is not a part of the yield effect). The column heading has now been changed to 'Displaced corn' and the text discussing the two tables (8 and 9) has been modified accordingly.
63	LH	4.2	Tables 8/9	ge	Explaining the differences between the states would help (e.g. why one require more inoculant, fieldwork, etc.…)	Briefly explain what the differences between the states are due to.	A brief explanation has been added as the second last

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							paragraph in Section 4.2.
64	LH/ MH	4.2	Table 8 and 9	te	Great to have the "yield effect" detailed. Yet, when dividing it by the additional corn (E.g. 0.041 Mg for Table 8), it does not match exactly the reference continuous corn profile (the difference is above 25% for K fertilizer; so it is more than a rounding effect). In section 4.3, page 29, it is mentioned that the displaced yield is from continuous corn (without P.b.).	Check that the inventory used for "yield effects" is the correct one.	The apparent inconsistency is in fact due to rounding. More significant digits have now been added. For K fertilizer (in Table 8), the emissions per Mg of corn can now be estimated as -0.15 kg CO_2e / 0.041 Mg = 3.76 kg CO_2e/Mg. This corresponds to the value for reference corn of 3.8 kg CO_2e/Mg (which has been rounded from 3.75).
65	NF	4.2	Table 8	ge	Why are methane and NO emissions excluded from the LCA?	Clarify	NO is not excluded from the LCA but it is not a greenhouse gas and hence not included in Table 8 (neither in Table 9). The omission of methane has been clarified in Section 4.1. Hence, no further clarification has been added.
66	MH	4.2	Table 8 and 9	ed	Header of last column: (Ref.¶ vs. Total P.b.) should rather be: (Total P.b. vs. Ref.¶)?	Check and correct if needed	Header of last column in Table 8 and 9 corrected as suggested by reviewer.
67	MH	4.2	3rd. para	te	You state that: "This aspect has however been ignored in the base case analysis by the assumption of no change in SOC in the displaced corn production (cf. Table 8 and 9). Thereby, it is only the impacts from avoided inputs (and their associated emissions) that are considered – not the land saving effect." Yet, there is an important "Yield effect (displacement)" negative CO_2 emission in Table 10?	Please clarify what it then is that is ignored here?	The yield effect in Table 10 relates to all changes in GHG emissions resulting from displacement of corn production elsewhere (driven by the yield increase on the inoculated corn field) – incl. the displaced inputs to corn production (elsewhere). Meanwhile, Table 10 ignores the GHG benefits from the

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							indirectly prevented/delayed land conversion. Thereby, it is only the impacts from avoided inputs (and their associated emissions) that are considered – not the land saving effect. The text has now been modified in attempt to spell this out more clearly.
68	MH	4.2	3rd. para	ed	You go on to state that “This can be considered conservative in the sense that it does not favour the P. bilaiae inoculant.” This is fine but in the tables you still present the absolute difference and only the relative difference is stated as N/A. This is a confusing for the reader and you should consider how to present the results in the table in accordance with what you explain here about the unknown effects of the system expansion on the affected lands. Maybe a footnote under the table briefly describing what is done and referring to the text for the explanation	Consider and revise as needed	The reason the relative difference has not been stated (or shown as ‘not applicable’) is that the GHG emissions from SOC in the reference system are negative. Hence, it is not meaningful to indicate a relative change (absolute change in SOC emissions divided by the SOC emissions in the reference system) as this would give a positive number, indicating that SOC emissions in the inoculant system are higher than in the reference system (which is not the case). A footnote has been added under the table (as suggested by the reviewer) to clarify this.
69	MH	4.3	Para 2	te	You state that: “The inputs to soybean production as well as the output (the soybeans) cancel out when the reference system is ‘subtracted’ from the inoculant system.” This is because the yield of soybeans is assumed not to be affected of whether the inoculant is used on the corn crop grown on the field the previous year or not. This should be explicitly mentioned and justified. It is not self-	Please make this clear in the text	The specified assumption has now been made clear in the text.

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					evident as the use of the inoculant might have lasting effects on the availability of P (e.g. by mobilizing it or affecting the labile fraction of P in the soil)		
70	LH	4.3	Para 3, 3rd line, page 28	ed	Mistyping: “Note that the relative difference in is ….”	Remove “in”	Now removed
71	LH	4.3	-	te/ge	Time perspective: the study involves a lot of issues related to time (crop rotation over 1-2 y, soil C modelling, “field” emission modelling, P status in the soil, etc.). See e.g. discussion page 29. Why did the authors not decide to incorporate time in the functional unit, so the time issues could have been dealt with uniformly? Using a certain amount of ha for corn production for 40 years, for instance? This would avoid all the proxy described on page 29.	Mention the possibility of alternative approaches, where relevant, to deal with time issues in a more consistent manner throughout.	The purpose of the study was to estimate the change in impacts per Mg corn when the *P.b.* inoculant is introduced. Hence, the functional unit was not tied to a specific area cultivated (as this would be determined by the yield). Note however that Equation 5 allows for an area-based calculation of the combined upstream and field effects in terms of global warming. That said, incorporating time and area into the functional unit would not solve the issue of changes extending beyond the cultivation period for corn in a corn-soybean rotation. This was solved by consideration of the changes in field emissions extending into the soy cultivation period. This was not inconsistent. It was simply a way to account for the full effect of the inoculant. As for the discussion on page 29 (in the draft report), this is only a question of finding a reasonable benchmark. The selected

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					Follow-up comment: OK (no further action required, but let me answer this comment): I fully acknowledge the challenge of dealing with time in LCA and dynamic inventories overall, and do support the approach of including the changes extending into the soy cultivation period. I had, however, understood		benchmark has no implications for the absolute changes in impacts. It only serves to put the absolute changes into perspective. As for the changes in SOC, there is no scientific consensus on how best to handle this. Hence, the issue was dealt with by exploration of alternative approaches (different time perspectives and a different method). It is hard to see how we could be more consistent on time issues and still remain true to the purpose of the study. In fact, we are not aware of other LCA studies that have consistently solved the issue of yield changes for one crop type (in this case corn) within a crop rotation including other crops, also (but happy to be enlightened). Hence, the decision has been not to make any revision in the report based on this comment (#71). Response to follow-up comment: We thank the reviewer for the follow-up comment – and wonder if there is in fact disagreement. We are looking at an area treated

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					that the authors did have the wish to tie the results to a specific area being cultivated (here Minnesota & North Dakota used to extrapolate conclusions to the whole of USA) – but indeed the additional text put in the limitation (2.1.6) section now clarifies this. Of course, if the ambition is to obtain the environmental performance per kg of (generic) corn produced with and without P.b., then incorporating the area and time in the functional unit won't make it; but the different time periods used to distribute some emissions (equally) over time (20y for SOC and 40y for substance flows related to "field activities") remains disturbing, from a scientific point of view. And no, I am not aware upfront of studies dealing specifically with yield increases in time for a given crop of interest within a certain crop rotation; although I am aware of a few efforts being undertaken to improve the consistency of including time dynamics in LCAs and Integrated Assessment Models (IAMs). This being said, I disagree that "incorporating time and area into the functional unit would not solve the issue of changes extending beyond the cultivation period for corn in a corn-soybean rotation": it is the whole point of looking at land as an area, and what happens to that area (and eventually others in the background system) for a certain period of time.		with the inoculant and considering what happens to this area (the field effect – incl. the part extending into next year's crop) and other areas affected (through the displacement of other crops). We are considering a certain amount of corn (1 Mg) – and the yield thereby determines the area we are considering (in the foreground system). This is the area called A in Fig. 1 and Fig. 2. One of the points made above was only that we could not specify the functional unit in terms of absolute values for quantity of corn *and* area because the relation between these is determined by yield, which differs from state to state.
72	LH	4.3	Table 10	te	Yield effect: the values shown for continuous corn are not the same as those in Tables 8-9. Follow-up comment: OK – but to make this absolutely clear I would suggest a footnote in Table 10, specifying that "the yield effect does not include changes in post treatment"	Check the "yield effect" values reported here.	The inconsistency occurred due to an incorrect header in Table 8 and 9 (the yield effect does not include changes in 'post treatment'). This has now been corrected. Decision on follow-up comment: Suggestion adopted
73	MH	4.3	Table 10	te	Yield effect: Not clear to me what this covers apart from the change in SOC in the saved land, which you	Please explain	The yield effect covers all impacts avoided due to

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					previously wrote that you ignore due to the uncertainties in assessing it?		displacement of crop production elsewhere (resulting from yield increases on existing cropland) – but land use-related GHG benefits (from reduced land occupation) are omitted in the base case results. This has now been specified in a note beneath the table.
74	NF	4.3	Table 10	ge	Is there a statistically significant difference between the modelled outputs	Clarify	Uncertainty of the results (including a discussion of potential statistically significant difference) has been addressed in Section 5.1.1.
75	LH	4.3	Table 11	te	There are reduction in eutrophication similar to those in global warming. But nowhere is it explained why it is so. Follow-up comment: OK – fine, as the reasons for the reduction in nutrient losses are now better explained in section 4.1. No further action	Explain, where relevant, what the reductions in eutrophication are due to.	As stated beneath Table 11, there was a substantial field effect from *P. bilaiae* for global warming and eutrophication. The latter is primarily explained by the reduction in nitrate losses to the aquatic environment (due to higher uptake of nitrogen in the corn) specified in Table 5 and 6. The text now makes reference to Table 5 and 6 in order to explain the observed field effect for eutrophication.
76	LH	4.3	Table 11	te	Acidification: it is counter-intuitive that the delta field is	Explain, for acidification, why the "yield effect" is	This has now been explained

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					positive, but the yield effect (not producing 0.041 or 0.044 Mg continuous corn) is overly negative (all 4 cases). Can this be explained anywhere?	so important compared to the field effect.	at the end of Section 4.4.
77	MH	4.4	Para 2	te	You state that there was a There was a significant field effect from P. bilaiae for global warming and eutrophication. “Significant” indicates statistical significance, but there has been no uncertainty analysis or statistical tests to support that the found differences for these two impact categories are indeed statistically significant	Use “substantial” or similar instead of “significant” or make clear that there is no statistical test to substantiate the significance	‘Significant’ changed to ‘substantial’
78	MH	4.5	Para 1	ge	Not clear why you want to develop a simplified formula when you have the detailed data for the two plots	Introduce the purpose of the simplified formula (upscaling to the rest of US?)	As mentioned in the text, the simplified formula has been derived to broaden the applicability of the study. The inoculant LCA represents a massive effort. If results were only applicable to corn in two US states, the efforts would be of very narrow use. But since results build on generic mechanisms (nutrient mass balances, etc.), a general formula would allow for assessment of other field trial results than those presented by Leggett et al. (2015). This means that farmers, research communities, and other stakeholders could conduct a quick assessment of GHG impacts related to observed yield results in specific field trials. This has now been embedded in the text to further specify the purpose the simplified/general formula.
79	MH	4.5	Para 5	te	“The 24.1 kg CO_2e Mg^{-1} is the average field effect for global warming (CO_2 and N_2O) in the base case (cf. Table	Not clear how this value is calculated from the numbers in Table 10 – please explain	The calculation has now

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					10).” - Corresponding to the average yield increase of 0.4 Mg ha^{-1}?		been specified in a footnote
80	MH	4.5	Para 6	te	“The average GHG impact for corn cultivation in the reference systems in the present study (Minnesota and North Dakota) is 312 kg CO$_2$e Mg^{-1} – based on N$_2$O emissions and ‘Inputs to the field’ in Table 8 and 9” - Not clear how this number comes out of Table 8 and 9. Also not clear how differences between crop-rotation schemes are represented?	Please explain	Explanation provided in footnote in the form of calculation example for Minnesota.
81	MH	4.5	Para 8	te	“…the 0.232 kg CO$_2$e ha^{-1} is the average difference in field emissions (N$_2$O and CO$_2$) between inoculated and non-inoculated corn production listed in Table 5 and 6 (converted to CO$_2$ equivalents).” - Not clear how this number comes out of Tables 5 and 6?	Please explain	There turned out to be an error in Equation 5. The text should read ‘232 kg’ (not ‘0.232 kg’). This has now been corrected. Besides, a calculation example for Minnesota has been added as a footnote so the reader can better understand how the average number for the four cases (232 kg CO$_2$e ha^{-1}) has been derived.
82	LH	4.5	Last para, line 9, P.32	ed	Mistyping: Effect if	Correct: effect of	Corrected
83	LH	4.5	Last para	te	Rather than 24.1 kg CO$_2$e Mg $^{-1}$ average field effect in the base case, I get 24.75, based on Table 10.	Check that the figure is correct.	The figure has been checked. The precise average of the yield effect in Table 10 is 24.075 kg CO$_2$e/Mg. This has been rounded to 24.1 CO$_2$e/Mg (and remains unchanged).
84	MH	5	Para 2	te	The criteria that you consider in the evaluation of the data quality and importance are not given anywhere	Please introduce these criteria to allow the reader to understand what is considered in the evaluation and agree or disagree with your evaluation	The criteria underlying the evaluation of the data quality and importance have now been discussed at the start of Section 5.
85	LH	5	Table 12	ed	Forgotten table note for “data quality”	Remove the note or explain it	Note removed

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86	MH	5.1	Para 1	te	Why use the simplified formula for investing the importance of the uncertainty given by the confidence intervals when you have all the data for these two fields? Why not redo the LCI and LCIA calculations with the values that represent the confidence interval limits?	Please explain	The simplified formula generates global warming results that are very close to those from the full LCA. In fact, it generates exactly the same results (by design), if state-specific and rotation specific values are entered. Hence, it was more efficient to rely on the simplified formula to illustrate the implications of the yield increase uncertainty than to redo the entire LCA with to different set of numbers. Meanwhile, the previous Section 5.1 has now been deleted and replaced with a more complete uncertainty assessment (to accommodate other comments/requests from the review panel).
87	MH	5.1	All section	te	You discuss uncertainty related to the yield increase using the provided confidence interval but I miss a discussion of uncertainties accompanying some of the other key data in the study. What are the uncertainties accompanying the results of the Day Cent model and the SOC modelling, in particular the emissions of CO_2 and N_2O from the soils?	Please address these uncertainties as well in your discussion of what are significant differences between the system with and without P.b. treatment	Uncertainties accompanying yield increase data and some of the other key data in the study have now been discussed in the new version of Section 5.1 (cf. response to comment 86).
88	MH	6	All chapter	te	I miss a sub chapter on uncertainty analysis discussing and quantifying, where possible, uncertainties on all the main contributing elements, not just the uncertainty of the yield estimates	Please identify and address other main sources of uncertainty as well in your discussion of uncertainties	Main sources of uncertainty have now been identified and addressed in the new version of Section 5.1 (cf. response to comment 86).
89	LH	6.2	Fig.5	te	Confusing figure. In the caption, the title "reduction" is confusing, as the effect is not the same for CO_2 and N_2O.	Harmonize the figure and the caption (vs not all emissions are reduced)	Fig. 4 and 5 in the draft report (now Fig. 5 and 6 in

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					I read this figure as more CO_2 emissions are obtained as the root% increases, though I know it is the opposite. I would suggest to present it the other way around (0% as reference, and the total emissions of other root% are smaller)		the revised report) have been changed so that they are now showing 'change' in GHG emissions (i.e. negative emissions) instead of 'reductions' in emissions. The two figures are thereby more in line with the results in Table 8-11 and Table 13-15. We hope this reduces the confusion mentioned in the comment.
90	NF	6.3	All	ge	Would be interesting to see a how sensitive the modelling results would have been to changes in some of the input assumptions made, including land use history etc.	Please comment on this	We agree that this could be of interest but the DayCent modelling team doubts that it will have a substantial influence on the difference in results between the reference systems and inoculant systems (i.e. the results that matter for the conclusion of the LCA). Hence, we have decided to forego sensitivity analyses of land use history but has mentioned this as a recommendation for future research in Section 8.3.
91	NF	6.3	6.3	ge	Petersen et al seem to recommend using soil C estimates to 1m in depth. How does this impact on the simulations by DayCent both in the sensitivity analysis but also the previous sections? Follow-up comment: "Out of scope" would be a better term than "irrelevant"	Statement in additional work/ short comings of modelling approach Consider and change if you agree	DayCent cannot estimate SOC change past 20cm. The implications of this shortcoming have now been discussed at the end of Section 6.3. Decision on follow-up comment: The term irrelevant has been

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							maintained. If changes were substantial, they should have been counted (‘in scope’) but, since changes are expected to be insignificant, they are considered irrelevant.
92	LH	6.4	Para 1	te	I disagree with the last sentence “land that may not be converted to agriculture”. We rather talk about land that is already under agriculture, but taken off the production (least competitive agricultural land)	Adjust the sentence	The global agricultural area (as a whole) is still expanding (while contracting in some regions, e.g. the EU). If yields can be raised on existing croplands, some of the ongoing expansion (at the frontier between nature and agriculture) can (indirectly) be prevented. The sentence has now been supplemented with a footnote to clarify the logic. In addition, a reference for has been made Kløverpris and Mueller (2013) for further discussion.
93	LH	6.4	Page 39, Para3	te	I do not follow your calculation. I would, e.g. for Minnesota, have done [1/(10.7+0.44) – 1/10.7] * 10,000 (ha needed P.b. minus ha needed ref). This would give a release of 37 m^2 for Minnesota and 49 m^2 for North Dakota.	Explain why you calculate the way you do.	We acknowledge that this calculation was not self-explanatory. The methodology section has now been slightly revised (Section 2.2.6.1 and 2.2.6.2 including Fig. 1 and Fig. 2) to explain the calculation – and a footnote in Section 6.4 now refers back to the explanation in the methodology section. Besides, there was an error in the text, which read 55 m^2,

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					Follow-up comment: OK. In footnote 30, it is written: "The relative change (reduction) in land occupation (per Mg corn) can be expressed as the area cultivated in the reference system (A in Fig. 1) divided by the area displaced in the inoculant system (B in Fig. 1)". I believe it should rather be the opposite (B/A).	Check and change if relevant	where it should have said 51 m^2. This has now been corrected (no impact on results, only a typo). Decision on follow-up comment: The mistake has been corrected. Well spotted!
94	LH	7	Par 1	te	Why do you use the average yield difference (0.4 Mg ha^{-1}) rather than the deviation of Leggett et al. here?	Explain the choice	We do use the deviation of Leggett et al. The 0.4 Mg ha-1 was a 'left-over' from a previous and less sophisticated version of the study. It has been deleted in the revised version of the report.
95	LH	7	Table 15	te	To back-calculate the figures, the reader needs to know the Mg corn needed per MJ ethanol Follow-up comment: OK- but relating to comment 96, it would help to have an example (e.g. as footnote) for the calculation of the "yield effect via ethanol production"	Provide the figure Mg corn needed per MJ ethanol Include an example of calculation for "yield effect via ethanol production"	Applied ethanol yield from corn (425 l Mg^{-1}) added to Table 15. Decision on follow-up comment: A calculation example has now been added in a footnote.
96	LH	7	Table 15	te	I would expect the "yield effect via ethanol production", representing the ethanol impacts per Mg corn and the gasoline replacement to be the same for both states, since the emission factors used for these are generic an not state-dependent. Follow-up comment: OK, but as per comment 95, a calculation example of the yield effect would makes this explanation even clearer	Provide an explanation for how yield effect is calculated.	An explanation has now been added as the second paragraph beneath Table 15. Decision on follow-up comment: A calculation example has now been added in a

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							footnote.
97	LH	7	Para 2	te	It is mentioned that using the corn obtained from the extra yield for the production of bioethanol gives “the most attractive results”. This statement is a half-truth if not supplemented. First, it must be highlighted that the availability of more “low iLUC risk” feedstock for bioethanol may involve less deployment of other technologies, say electric vehicles (and the statement only applies providing gasoline is the fuel displaced). Second, in table 14, one of the iLUC methods leading to the lowest GHG estimates is used. It is rightly highlighted in p.40 that results could have looked (very) different with another method, and likely this would have been in one direction, increased savings. In such case, differences between using the extra corn for ethanol or for reducing the pressure on land would be smaller, if not in the other direction.	Supplement the statement.	A discussion of other ILUC results has now been added in Appendix F. In addition, the text in Section 7 has now been supplemented to point out that, in case of higher ILUC estimates, the alternative scenario with ethanol might not yield the best GHG results. It is beyond the scope of the present report to enter a discussion of whether “low ILUC risk” feedstock for biofuels could involve less deployment of other technologies such as electric vehicles (EVs). We only note that, even with the most aggressive projections of EV penetration (and parallel expansion of green electricity capacity), there continues to be a massive challenge in reducing GHG emissions from transportation – and, even in the light duty vehicle sector, there will continue to be a massive demand for liquid fuels for decades. On top of this comes the demand for heavy duty transport and aviation. In that light, it is considered reasonable to assume that liquid biofuels will displace liquid fossil fuels in general

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					Follow-up comment: OK for the first part- references cited in Appendix F must be added to the reference list. Here, it is the wording "…gives the most attractive result…" that makes me uncomfortable. For instance, there are additional ways of using the "low iLUC" risk feedstock not investigated in this report (e.g. feedstock for biogas plants; renewable gas can also be used to mitigate emissions from the transport sector).	Please rephrase in a way that clearly mentions that according to Table 15, the use of yield increase from P.b. to produce ethanol presents a greater performance than its use for relieving pressure on land, in terms of global warming. (of course keeping the statement on a higher ILUC factor afterwards)	and that ethanol will displace gasoline in this specific case (also considering the fact that ethanol is an internationally traded commodity). Decisions on follow-up comment: The missing reference for Woltjer et al. (2017) has now been added to the reference list. It has now been specified that the ethanol option gives the most attractive global warming results *out of the options investigated in the present study* (implicitly indicating that there may potentially be other and even more attractive options).
98	MH	8		te	I miss a discussion somewhere of potential negative impacts of P. bilaiae not covered by the study, e.g., permanent change of microflora in the soil with other possible consequences. I also miss an explicit consideration of whether there might be other environmental impacts that are not addressed properly by the LCIA	Please address these points in the interpretation	We have now added a section in the Impact Assessment chapter that considers potential impacts on microflora, toxicity, and use of water resources.
99	NF	8.1	Para 1, line 1	ge	Experimental data mentioned indicated that yield gains were only statistically significant in one region. If modelling approach indicates for both that there are gains (which on average there seems to be), some quick stats between the datasets would confirm this statement.	Please check and comment on this	The reviewer has withdrawn the comment…
100	LH	8.1	Para 1	te	"no observed trade-offs" is mentioned (as in the summary). Again, this could be a misleading half-truth if not supplemented (e.g. for the impact categories studied).	Supplement the statement.	The statement has been supplemented as suggested by the reviewer.

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101	MH	8.1	Para 1	te	"No observed trade-offs" - There are trade-offs but the net result is an improvement for all studied indicators (so no net trade-offs between environmental impact categories)	Clarify	It has now been clarified that no net trade-offs have been observed.
102	MH	8.1	Para 5	te	When concluding on the uncertainty and (indirectly) on the significance of the found difference between the system with and without treatment, you must also comment on other sources of uncertainty and their ability to affect the results – notably the uncertainties of the DayCent model estimates.	Preferably, these uncertainty sources should be treated in a sub chapter on uncertainty analysis. Anyhow, they need to be mentioned in the discussion of the findings and the conclusion.	A sub chapter on uncertainty analysis has now been added (new version of Section 5.1.1) and the uncertainty sources have been mentioned in the conclusion.
103	MH	8.3	Third bullet	ed	"(for Minnesota as well as for Minnesota)" not meaningful	Amend sentence	Sentence deleted (cf. response to comment 41)
104	NF	8.3	Pg 44, final statement	ge	While the modelling study points to some potential the final statement overstates, based on the work to date, the widespread benefits of the inoculant.	Amend statement	The statement has been amended to specify that it applies to US corn (based on the calculations in Appendix D, which shows that the upstream effect (based on a worst-case estimate) only reduces the total GHG savings by less than 4%.
105	NF	9	Alexandrato et al	ed	Change from 2012 to (2012)		Requested change adopted
106	NF	9	Grant et al	ed	Change from 2016 to (2016)		Requested change adopted
107	NF	9	Leggett et al	ed	Check year		Year corrected (cf. response to comment 11)
108	LH	Annex A	Para 2	te	Values from Franzen (2010) are presented, in t ha^{-1}. Yet, the report overall uses values in Mg ha-1, to avoid confusion with the imperial ton.	Harmonize to Mg ha-1 and double-check that the values retrieved from Franzen (2010) are really for metric tonne (and not imperial, as used in the US)	Value has been double-checked and the unit has been harmonized with the rest of the report.
109	LH	Annex A	Para 2	ge	The following is mentioned "Based on five North Dakota field trial reports, we estimate …". It would be more convincing if you implicitly mentioned these reports.	Insert the appropriate references.	The five data points mentioned were part of the field trial information compiled and published by

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							Leggett et al. (2015). This has now been specified in a footnote.
110	LH	Annex A	Para 3	ed	Potassium is mentioned twice in this paragraph, where one should rather read phosphorus.	Change “potassium” for “phosphorus” for these 2 occurrences.	The text reads correctly. It should indeed say potassium in the two instances mentioned. In order to help the reader, we have now added ‘Total P’ in a parenthesis first time phosphorus is mentioned and ‘Total K’ in a parenthesis first time potassium is mentioned.
111	NF	8.3	Third bullet (revised report)	te	While the DayCent results could be fine - tuned to match the reported yield deviations exactly this could mean that the model is over-calibrated to match the site conditions exactly, therefore upscaling to a regional or national level would be difficult.	Please comment	The concern of the reviewer has now been addressed in a footnote.
112	NF	8.3	Fourth bullet (revised report)	te	Further research related to DayCent modelling could also be the implementation of the effect of the inoculant within the model. Based on how DayCent currently works the modelling approach used here is sensible, however as the modelling approach aims to reflect the indirect effect of the inoculant, improvements should also focus on integrating how the inoculant works into DayCent itself. An additional sentence about the uncertainty of the approach could be added into point three of four.	Consider and amend as relevant	We find these proposals interesting but have decided to leave them out of the recommendations.

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