Influence of genetically modified organisms on agro-ecosystem processes

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ABSTRACT

Biotechnology offers extensive possibilities to incorporate new traits into organisms. Genetically modified (GM) traits relevant for agro-ecosystems include traits such as pest resistance and herbicide tolerance in crop plants, increased growth rate in fish and livestock, and enhanced nitrogen-fixation capabilities of soil microbes. In this review, we evaluated the direct and indirect trait-specific effects of GM plants, microbes, and animals on ecosystem processes and found that most of the effects of genetically modified organisms (GMOs) on ecosystem processes are indirect and are the result of associated changes in management strategy rather than a direct effect of the GMOs. Conflicting results on the performance and effects of GMOs are frequently reported, especially regarding crop yield and impacts on soil organisms. This is partly because methods with different levels of resolution have been used in different ecological contexts. Overall, there is little evidence that the effects of GM traits on ecosystem processes act with different mechanisms from those of traits modified using conventional methods. However, little is known about trait-specific effects of GMOs on ecosystem processes even though GMOs have been used for more than three decades. In particular, studies linking genetically modified traits to ecosystem processes at longer time scales are rare, but needed for evaluating trait effects, especially in an evolutionary context. In addition, biotechnology may provide a unique tool for gaining insights into the links between traits and ecosystem processes when integrated into basic ecological research.

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1. Introduction

Biotechnology can be used to modify traits in crops and animals much faster and more accurately than conventional breeding. Examples of genetically modified organisms (GMOs) include crop plants genetically engineered to express insect resistance genes or contain herbicide tolerance genes, bacteria engineered for enhanced nitrogen-fixing capabilities, and animals genetically modified for increased growth rate (Table 1). GMOs were first introduced into commercial agriculture in the USA in 1996, and have often led to more flexible and efficient management strategies (Zilberman et al., 2010). Trait manipulation of target organisms and production system components (Fig. 1) also creates opportunities for improved products obtained with more effective resource utilisation and reduced negative environmental impact. Nevertheless, manipulated traits may introduce unforeseen effects on ecological processes, and there is a possibility of trade-offs at the genetic, physiological or ecological level that constrain the opportunities for improving resource-use management using modern biotechnology (Weih, 2003). The functional traits of dominant species in an ecosystem are known to have strong effects on ecosystem processes and services (de Bello et al., 2010; Grime, 1998). Thus, the adoption of novel transgenic plants, animals, and microbes in agricultural systems globally may have potentially large impacts at ecosystem level.

In this review, we give a comprehensive overview of the direct and indirect effects of GMOs, including plants, microorganisms and animals, on agro-ecosystem functioning in terms of: net production; nutrient cycling; greenhouse gas fluxes; biodiversity; and crop-weed and trophic interactions (Fig. 1). First, we summarize the current knowledge of effects of GMOs on the above listed ecosystem processes and then we discuss knowledge gaps and provide suggestions on how to integrate GM traits into basic ecological research. The examples of GM plants, microorganisms and animals discussed here are either currently in use or most likely to be of practical use for land-based food, feed, fibre and bioenergy production in the near future. GMOs used for medical purposes were not considered, since they are not currently produced in natural or open ecosystems.

2. Documented direct and indirect effects of GMOs

2.1. Net production

Yield of maize, wheat, rice and soybean has stagnated or collapsed over the last 50 years in several areas of the world, causing yield gaps compared with expected production (Ray et al., 2012). Agricultural management strategies that increase net crop production are therefore needed (Mueller et al., 2012; Ray et al., 2012), especially as climate change is expected to alter growing conditions worldwide changing the prerequisites for agriculture. Plant production can be enhanced directly by improving intrinsic yield, or indirectly through the reduction of pest, pathogen and weed pressure. GM crops engineered for herbicide tolerance (HT), pest resistance and/or improved stress tolerance are expected to meet part of the demand of increased production (Areal et al., 2013). In climate chamber experiments, rice engineered for improved nitrate uptake performed better than non-modified rice under both optimal and low nitrogen availability (Ranathunge et al., 2014) although results remain to be replicated in the field. Both field and mesocosm experiments have demonstrated that the interaction between the environment and the trade-off between traits is of importance for evaluating effects on net production. For example, trade-offs between pest resistance and drought tolerance (Naik et al., 2005; Vanni et al., 2010), pest resistance and nitrogen use efficiency (Haegel and Below, 2013), and stacked traits and fitness (Londo et al., 2011a; Shi et al., 2013) have been studied in different environmental settings, making results difficult to generalize. Hence, environmental factors such as temperature and precipitation (Naik et al., 2005; Shi et al., 2013; Xu et al., 2013), in combination with crop varieties and traits, cause significant differences in estimates of expected yield between studies (Alston et al., 2009; Edgerton, 2009; Xu et al., 2013). In a 20-year field experiment, Shi et al. (2013) documented increased yields in maize cultivars producing toxins from Bacillus thuringiensis (Bt) targeting the European corn borer (Ostrinia nubilalis) or conferring glufosinate-tolerance, but not Bt cultivars targeting corn rootworm (Diabrotica sp.) or conferring glyphosate-tolerance. Yearly variation in yield was lower in the transgenic cultivars than in the conventional cultivars highlighting the importance of long-term studies.

GM microorganisms can be tailored to increase plant production. For example, a strain of the nitrogen fixing bacterium Sinorhizobium meliloti, engineered to overexpress genes involved in establishment of symbiosis with lucerne (Medicago sativa) was shown to have a transient advantage, both in microcosm and field experiments, when compared with non-modified strains (van Dillewijn et al., 2001). When this GM strain was evaluated in the field, S. meliloti rapidly colonized the rhizosphere of its host plant and impeded growth of Pseudomonas (Schwieger and Tebbe, 2000), demonstrating that the microbial community in the rhizosphere can respond quickly to the introduction of microbial strains. However, the influence of the plant species itself was greater than the effect of inoculation of GM S. meliloti on the microbial community composition (Schwieger and Tebbe, 2000). Overall, the limited knowledge that exists on use of GM microorganisms in the field makes it difficult to draw any firm conclusions regarding their ecological consequences and effects on net production.

GM farm animals and fish for food and feed include species genetically modified to improve traits of economic importance (Table 1; Forabosco et al., 2013), such as growth rate (Devlin et al., 2001), milk composition (Reh et al., 2004), disease resistance (Wall et al., 2005) and survival (Konishi et al., 2011). Biotechnological modifications of non-domesticated animals have been made primarily in fish to increase growth rate. The body weight of GM salmonids, carp and tilapias can be up to 100 times that of the wild type at the same age (Nam et al., 2008). The AquAdvantage® salmon developed by AquaBounty Technologies is an Atlantic salmon (Salmo salar) with a gene from Chinook salmon (Oncorhynchus tshawytscha) and a promoter from ocean pout (Zoarces americanus), which allowed the GM fish to grow 5–10 times faster than their non-GM counterparts during the pre-smolt stage. Hence, these GM fish reach market size one year earlier than non-GM salmon (Butler and Fletcher, 2009; Fletcher et al., 2004). Another strategy to increase production is through manipulating the muscles to produce two muscle layers instead of one and this has been accomplished in rainbow trout (Oncorhynchus mykiss) (Medeiros et al., 2009).
Table 1 Examples of genetically modified traits and organisms with relevance for food, feed, fuel and fibre production. For plants, only deregulated crops and crops at the field trial stage are included.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Modified trait</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants</td>
<td>Increased biomass</td>
<td>Hybrid aspen</td>
<td>Oh et al., 2011</td>
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<td></td>
<td>Product quality</td>
<td>Maize, Oilseed rape, Soybean, Cassava</td>
<td>Franchi et al., 2004</td>
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<td></td>
<td>Insect resistance</td>
<td>Cotton, Maize, Poplar, Rice, Soybean, Tomato, Potato</td>
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<td></td>
<td>Virus resistance</td>
<td>Bean, Papaya, Plum, Potato, Squash, Sweet pepper, Tomato</td>
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<td></td>
<td>Drought tolerance/resistance</td>
<td>Maize, Hybrid aspen, Wheat</td>
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<td></td>
<td>Increased/ altered nutrient uptake</td>
<td>Wheat, Barley</td>
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<td></td>
<td>Fortified food</td>
<td>Tomato, Strawberry, Potato, Rice</td>
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<td>Delayed ripening (Shelf life)</td>
<td>Melon, Tomato</td>
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<td></td>
<td>Pheromones</td>
<td>Wheat</td>
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<td>Anti-allergy</td>
<td>Rice</td>
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<td>Nicotine reduction</td>
<td>Tobacco</td>
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<td>Altered phosphorus metabolism</td>
<td>Oilseed rape, Maize</td>
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<td>Nopaline synthesis</td>
<td>Lineseed</td>
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<tr>
<td>Microorganisms</td>
<td>Increased biomass production (direct)</td>
<td>Cyanobacteria, Rhodobacter sphaeroides, Brevibacterium lactofermentum, Saccharomyces cerevisiae</td>
<td>Oh et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Increased biomass production (indirect)</td>
<td>Sinorhizobium meliloti</td>
<td>van Dillewijn et al., 2001</td>
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<td></td>
<td>Enhanced N₂O reduction</td>
<td>Bradyrhizobium japonicum, Nitrosomonas europaea</td>
<td>Subbarao et al., 2006, Itakura et al., 2013, Itakura et al., 2013</td>
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<td></td>
<td>Immobilization of heavy metals in soil</td>
<td>Ralstonia eutropha</td>
<td>Valls et al., 2000, Ripp et al., 2000, Haro and de Lorenzo, 2001</td>
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<tr>
<td></td>
<td>Enhanced bioremediation</td>
<td>Pseudomonas sp.</td>
<td></td>
</tr>
</tbody>
</table>

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**a** Sources: Information Systems for Biotechnology database (http://www.isb.vt.edu), Swedish Board of Agriculture (http://www.jordbruksverket.se/amnesomraden/odling/genteknikgmo/faltforsok.4.300b18bd13d103e79ef80002497.html), and EU register of authorised GMOs (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm).

**b** Modified amino acid/protein, oil/fatty acid, starch/carbohydrate.

**c** Iron, zinc and/or vitamin C fortified.

**d** Phytase reduction, catalyses inorganic P to digestible organic P in grains and oilseed. Phytase reduction, reduces P in animal waste and improves uptake of micronutrients in food.

**e** Nopaline is used as a marker in plant breeding.

**f** The biomass is a direct product of the genetically modified microorganism.

**g** The biomass is synthesised by plants with enhanced productivity due to intensified symbiotic interactions with genetically modified, beneficial microorganisms.
2.2. Nutrient cycling

Nutrient cycling is directly affected by modification of genes involved in plant nutrient use and indirectly by affecting the decomposition of organic matter, e.g. by modifications of metabolites or plant tissue composition. In terrestrial ecosystems, no differences in decomposition rates of residues have been found for Bt maize (Yanni et al., 2010), Bt rice (Fang et al., 2012; Lu et al., 2010a) or wheat with antifungal properties (Duc et al., 2011) when compared to their non-GM counterparts in field experiments. There were also no differences in decomposition rate between transgenic and non-transgenic Populus leaf litter (Axelson et al., 2011) or Bt and non-Bt maize plant material in aquatic ecosystems (Chambers et al., 2010; Swan et al., 2009). Agricultural practice (Londoño-R et al., 2013; Mungai et al., 2005) and type of plant tissue (Londoño-R et al., 2013) have been identified as being more important for decomposition rate than GM or non-GM cultivar in field studies. However, a field experiment using a transgenic birch line with antifungal properties showed a slight increase in nematodes feeding on bacteria rather than on fungi, although there were no effects on the rate or quality of the decomposition process (Vauramo et al., 2006). This can be interpreted as a reduced amount of fungi on the leaves because of the antifungal properties of the birch, and hence less food for fungivorous nematodes. Changes in the microbial community composition in the rhizosphere of GM and non-GM maize grown in the field have been reported, although differences were not greater than those between conventional varieties (Dohrmann et al., 2013).

Indirect effects on nutrient cycling mediated by microorganisms can also be caused by the use of glyphosate on HT crops, since this herbicide contains both carbon and nitrogen. In a laboratory study using nine different soils, the addition of glyphosate exerted a strong effect on nitrogen cycling, both in terms of increased amount of nitrogen stored in microbial biomass and long-term enhancement of nitrogen mineralisation (Haney et al., 2002).

Indirect effects on nutrient cycling can also occur as a result of alterations to nutrient stoichiometry in the rhizosphere. In a pot experiment with Bt cotton, availability of ammonium and nitrate in the rhizosphere was reduced and phosphorus availability was enhanced compared with the isogenic line (Sarkar et al., 2008). Leaves of Bt cotton plants have been found to harbour higher amounts of nitrogen in the form of amino acids and free proteins than leaves of non-Bt plants (Chen et al., 2005). This suggests that Bt cotton reduces the amount of available nitrogen in the rhizosphere by increased uptake and incorporation of nitrogen from ammonia and nitrate in the leaves compared with the isogenic line. Alternatively, a modified plant with exudates enriched in organic acids may decrease pH in the rhizosphere, reducing the rate of nitrification (Motavalli et al., 2004), which can impact the cycling of soil N.

Livestock is the single most significant contributor to nitrogen and phosphorus pollution worldwide (Sutton et al., 2011) and efficient feed utilisation in GM animals has the potential to enhance nutrient cycling by reducing losses of nutrients from the animal to the manure. The GM pig Enviropig utilise all phosphate in grain and soybean and excrete 60% less phosphate than conventional pigs (Golovan et al., 2001). This is because Enviropigs, in contrast to conventional pigs, express the enzyme phytase in saliva and can thereby digest and extract much more of the phosphorus from plant feed. Therefore, supplementary phosphorus in the feed can be avoided, which reduces direct feed costs, as well as the environmental footprint of feed production. Phillips et al. (2006) showed that 33% less land would be required to spread manure from Enviropigs compared with conventional pigs. One criticism of the Enviropig is that it allows pig farmers to store more manure on farm, due to decreased phosphorus concentration in the manure. This can lead to environmental problems through increasing the concentration of other substances, such as nitrogen and ammonium (Food & Water Watch, 2010).
2.3. Greenhouse gas fluxes

GMOS can affect greenhouse gas (GHG) emissions directly by altering emissions that occur from plants, soil organisms and farm animals or indirectly as a result of management changes within the production system. Within forestry, poplar trees with low isoprene emissions have been developed to reduce GHG production (Behnke et al., 2012), but for arable crops, the reduction of GHGs is more indirect. The reduced spraying of insecticides associated with insect-resistant crops and the no-till practices associated with HT crops are estimated to have led to fuel savings of over 8000 tons of CO₂ between 1996 and 2008 (Brookes and Barfoot, 2010). However, increase in herbicide application has undermined the benefits of no-till practices regarding weed control (Mortensen et al., 2012), selecting for herbicide resistant weeds. A combination of farming practices using mechanical weeding and use of herbicides with different mode of action has been proposed as a way to reduce weed problems (Gilbert, 2013; Harker et al., 2012), but increased tillage counteracts the previous reduction in CO₂ achieved by the reduced use of fuels in the no-till system.

Reducing N₂O rather than CO₂ emissions from soil has been the focus of recent microbial research aimed at addressing options for GHG mitigation. Two microbial processes, nitrification (the oxidation of ammonia to nitrate) and denitrification (the reduction of nitrate to N₂O or nitrogen gas), are of particular importance for N₂O emissions. Biotechnological tools to improve the efficiency of denitrifying microorganisms with a complete process ending with N₂ rather than N₂O or to express bacterial N₂O reductases in plants have been discussed (Philippot and Hallin, 2011; Richardson et al., 2009). The former approach has recently been demonstrated by Itakura et al. (2013) in microcosms, and the latter in the laboratory (Wan et al., 2012). Crop plants can also be genetically modified to produce biological nitrification inhibitors (Subbarao et al., 2007, 2009), which can help to reduce N₂O emissions originating from nitrification and denitrification, as the substrate for denitrification is not produced when the nitrifiers are inhibited. Another beneficial aspect of such genetic alteration is that nitrate leaching may be decreased. Thus, recent research has demonstrated the feasibility of several strategies to use GM microorganisms to reduce N₂O emissions. The challenge will be to find ways to implement these approaches under field conditions.

Livestock contributions to the total anthropogenic GHG emissions are 9% CO₂, 37% CH₄ and 65% N₂O (Steinfeld et al., 2006). Livestock that increase productivity (i.e. with a better ratio between production and consumption per animal) can be expected to indirectly reduce GHG emissions in the animal product chain. Another strategy, directly targeting GHG emissions, is to reduce methane emissions from the rumen (Hegarty, 1999; Hook et al., 2010; Ross et al., 2013).

2.4. Increased or decreased biodiversity

The effects of GMOs on biodiversity have been discussed primarily in terms of the potential risk of species losses and the impact that a GM trait could have on the genetic diversity and subsequent ecosystem functioning in a given landscape. Field studies of HT oilseed rape, BT maize, BT rice, GM wheat with antifungal properties and GM potato with altered starch production have demonstrated that environmental and biotic factors, in addition to agricultural practices such as type of tillage system, can have a greater impact on soil microfauna and microbial communities than the genetic properties of the crop (Baumgarte and Tebbe, 2005; Duc et al., 2011; Dunfield and Germida, 2003; Griffiths et al., 2007; Hannula et al., 2012; Liu et al., 2008; Lupwayi et al., 2007; Sessitsch et al., 2004). No substantial effects of BT crops on earthworms (reviewed in Icoz and Stotzky, 2008; Shu et al., 2011), woodlice, collembolas, mites and protozoa (reviewed in Icoz and Stotzky, 2008), or non-target Chrysomelids (reviewed in Devos et al., 2012) have been detected in field and laboratory studies, and in microcosms no effects were observed on snails and microarthropods (de Vauclare et al., 2007). Axelson et al. (2011) found increased population densities of some aquatic insects in stream water containing Bt Populus leaf-litter, but were unable to identify any single variable that related to this shift in the faunal community. However, possible effects at species level on soil nematodes and non-target insect larvae have been reported (Icoz and Stotzky, 2008 reviewed in Cheeeke, 2012a). The leaf-shredding invertebrate Lepidostoma lida, found in freshwater streams, showed lower growth rate in the laboratory when feeding on debris from BT maize, but not in field situations. This was suggested to depend on additional stress factors in natural streams, such as pesticides and altered water flow (Chambers et al., 2010). For soil fungi, there is evidence from greenhouse studies of decreased colonisation by arbuscular mycorrhizal fungi in several lines of BT maize (Cheeeke et al., 2011, 2012b). This effect was not observed under field conditions (Cheeeke et al., 2013), although lower densities of arbuscular mycorrhizal fungi spores were detected in plots with a history of BT maize cultivation compared to plots with non-BT maize (Cheeeke et al., 2014). No negative effect of BT maize has been observed with respect to microbial community-level physiological profiles in both growth chamber (Blackwood and Buyer, 2004) and field studies (Lupwayi et al., 2007) or on nitrate reduction rate in the field (Philippot et al., 2006). Rice and cotton modified to express BT toxins have not caused observable shifts in soil bacterial and fungal communities in the field (Lachnicht et al., 2004; Li et al., 2011; Lu et al., 2010a) or in laboratory experiments (Shen et al., 2006). An indirect positive effect of BT crops on biodiversity is the reduced insecticide use (Benbrook, 2012; Naranjo, 2011), enhancing the density of non-target organisms (Naranjo, 2011). Although some of these non-target organisms have become pests, there are also examples were an increase in natural enemies to pest insects reduces pest pressure (Naranjo, 2011).

In an extensive field study, no clear correlation was found between HT beet, HT spring oilseed rape or HT maize and the abundance and diversity of different plant and invertebrate species (Hawes et al., 2003). Rather, the weed management strategies that accompanied HT crop cultivation affected the abundance of invertebrates and caused a shift from a herbivore to a detritivore-dominated food web. Similarly, effects of altered herbicide treatments as a consequence of adopting HT maize are reported to have led to reductions in milkweed populations, reducing the trophic structure of the plant community. However, possible effects at species level on soil nematodes and non-target insect larvae have been reported (Icoz and Stotzky, 2008 reviewed in Cheeeke, 2012a). The leaf-shredding invertebrate Lepidostoma lida, found in freshwater streams, showed lower growth rate in the laboratory when feeding on debris from BT maize, but not in field situations. This was suggested to depend on additional stress factors in natural streams, such as pesticides and altered water flow (Chambers et al., 2010). For soil fungi, there is evidence from greenhouse studies of decreased colonisation by arbuscular mycorrhizal fungi in several lines of BT maize (Cheeeke et al., 2011, 2012b). This effect was not observed under field conditions (Cheeeke et al., 2013), although lower densities of arbuscular mycorrhizal fungi spores were detected in plots with a history of BT maize cultivation compared to plots with non-BT maize (Cheeeke et al., 2014). No negative effect of BT maize has been observed with respect to microbial community-level physiological profiles in both growth chamber (Blackwood and Buyer, 2004) and field studies (Lupwayi et al., 2007) or on nitrate reduction rate in the field (Philippot et al., 2006). Rice and cotton modified to express BT toxins have not caused observable shifts in soil bacterial and fungal communities in the field (Lachnicht et al., 2004; Li et al., 2011; Lu et al., 2010a) or in laboratory experiments (Shen et al., 2006). An indirect positive effect of BT crops on biodiversity is the reduced insecticide use (Benbrook, 2012; Naranjo, 2011), enhancing the density of non-target organisms (Naranjo, 2011). Although some of these non-target organisms have become pests, there are also examples were an increase in natural enemies to pest insects reduces pest pressure (Naranjo, 2011).

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GM microorganisms have been applied in the field to a considerably smaller extent than GM plants, but are likely to be on the market in the future, especially in pest control applications. The potential environmental hazards associated with GM microorganisms are primarily related to the spread of GM traits, since bacteria and archaea are known to exchange genetic material even across distantly related species by horizontal gene transfer. The genetic stability of biotechnologically derived microorganisms
depends on whether the foreign genetic element has been introduced into the chromosome (Lilley and Bailey, 1997) or added on a plasmid (Drahos et al., 1986). When a presumably neutral marker gene cassette was introduced to a *Pseudomonas* strain on a plasmid, it could be retrieved from indigenous *Pseudomonas*, but not when integrated into the chromosome (Lilley et al., 2003). However, when GM *P. putida* cells with the target genetic element on a plasmid mated with bacteria extracted from soil, less than 1% of the indigenous soil bacteria could take up the plasmid (Shintani et al., 2014). This suggests that gene mobility through horizontal transfer may be limited in the environment. Other effects of GM microorganisms on indigenous microbial communities have been studied using *Pseudomonas fluorescens* engineered to produce antibiotics (Bankhead et al., 2004) and *S. meliloti* with added genetic markers or, enhanced competitiveness in both microcosm and field experiments (Andronov et al., 2009; Schwieger and Tebbe, 2000; van Dillewijn et al., 2002). While no effects on the indigenous soil microbial community were observed in the first two studies, the latter two reported an increase in the number of gammaproteobacteria (*van Dillewijn et al., 2002*) and rhizobia (*Schwieger and Tebbe, 2000*) after inoculation of the GM *S. meliloti*. van Dillewijn et al. (2002) made similar observations after inoculation of wild-type *S. meliloti*, but in an experiment by *Schwieger and Tebbe (2000)* crop type had a greater effect on the microbial community composition than the inoculation of marker gene-tagged *S. meliloti*.

Highly domesticated livestock such as cattle and pigs often have reduced fitness in natural environments, but there are examples with i.e. escaped sheep and horses having a profound impact on local environment on e.g. islands (Beltrán et al., 2014; Freedman et al., 2011). Non-domesticated GM animals with retained fitness in the wild may affect biodiversity if they escape from agro-ecosystems in which they are raised, if the modified genes confer a competitive advantage. An example is the transgenic salmon, with its enhanced growth rate and ability to consume more stream resources than the wild-type salmon, but the outcome is very difficult to predict (Sundström et al., 2010). Likewise, the release of GM insects to tackle dengue fever or malaria will most likely have effects on natural populations when releases are made at large scale (Marshall, 2010). In situations when field data is difficult to retain, modeling of different scenarios will be relevant as a complement in risk assessment (Li et al., 2015).

### 2.5. Crop-weed and trophic interactions relevant to pest management

Insect resistance to *Bt* crops and herbicide resistance in weeds is more likely to occur when GM plants with tolerance to a particular herbicide or insect pest are used without alternating with mechanical weeding or alternative pest-control strategies (Harker et al., 2012). Insect resistance to *Bt* toxin has developed in almost half of the 13 major pest populations targeted by *Bt* crops (Gassmann et al., 2014; Tabashnik et al., 2013). Monitoring for pest resistance and utilising integrated management strategies can enhance the success of *Bt* crops (Tabashnik et al., 2013) i.e. using various refugia strategies, e.g. targeting recessive resistance, releasing sterile pest insects to reduce reproduction of resistant individuals and mixed planting of *Bt/non-Bt* seeds within the same field (Monsanto, 2014a,b; Tabashnik et al., 2010). The need for such strategies is emphasised by surveys on field populations of e.g. cotton boll worm (*Helicoverpa armigera*) which has been found to harbour resistance genes to *Bt* toxin (Zhang et al., 2012). There are also reports of shifts in pest species on cotton as a result of reduced target pest pressure (Lu et al., 2010b; Zhao et al., 2011).

Glyphosate resistance in weeds has been detected in all countries where glyphosate is applied and at present, 30 weed species with populations being glyphosate-resistant have been reported to the International Survey of Herbicides (Heap, 2014). Gliddon (1994) and Ellstrand (2003) show that gene flow from conventionally bred crops to wild relatives has always existed, and hence gene flow between transgenic crops and wild relatives would be expected, but the ecological consequences will depend on whether specific traits result in increased, neutral or decreased fitness effects in the recipient populations (Ellstrand et al., 2013). Similarly, the intrinsic biology and management of the crops will influence the amount of gene flow. In oilseed rape, hybrids between HT oilseed rape (*Brassica napus*) and the weed wild turnip (*Brassica rapa*) have been found over a short time span (four years) in field borders (Warwick et al., 2008), despite a lack of selection pressure for HT and reduced pollen viability of the HT hybrids. Crop rotation targeting plant pathogens may provide a sufficient time span to reduce further pollen transfer from HT oilseed rape to wild turnip. However, oilseed rape is renowned for its long-term seed dormancy and volunteer GM plants, i.e. plants appearing in the following crop, can occur 10 years after GM oilseed rape cultivation (D’Hertefeldt et al., 2008). Effective and efficient control of volunteers is crucial in order to avoid build-up of oilseed rape seeds in the seedbank and continued occurrence of volunteer oilseed rape (Beckie et al., 2006; Begg et al., 2006). Volunteer GM plants can be controlled when tilled or sprayed at early growth stages, but control of glyphosate-tolerant volunteers requires spraying with more potent herbicides such as 2,4-D or phenoxy-based herbicides (Légère et al., 2006). HT oilseed rape has also been shown to disperse along transport routes, thereby establishing viable populations far from the original fields (Kawata et al., 2009; Schafer et al., 2011). In this feral context, these populations can act as a pool for transgene persistence (Squire et al., 2011) and there is a potential to increase gene flow of the HT trait although oilseed rape is mainly self- and insect pollinated and to a much lesser extent pollinated by wind (Ramsay et al., 2003; Warwick et al., 2008). The dispersal characteristics of oilseed rape varies significantly compared to the outbreeding and wind pollinated creeping bentgrass (*Agrostis stolonifera*), which has abundant pollen flow over large distances. This long-distance pollen dispersal is likely to be the cause of the rapid escape of HT creeping bentgrass from a field trial into natural adjacent habitats (Zapiola et al., 2008). Recently, spontaneous hybridisation between HT *A. stolonifera* and wild *Polygogn monspeliensis* has also been reported (Zapiola and Mallory-Smith, 2012). By using models that identify crucial steps in the crop lifecycle, gene escape from HT crops to weeds may be mitigated (Begg et al., 2006; Sester et al., 2012), and simulation models can also be used to study the evolution of resistance in weeds and to evaluate different management strategies (Bagavathiannan et al., 2014).

Microbial communities are continually being screened for use as biological control agents that can then be further enhanced using biotechnology. A commercial strain of GM *Agrobacterium radiobacter* (K1026) has been used successfully against crown gall-inducing agrobacteria (Vicedo et al., 1993). At the laboratory scale, researchers have successfully modified different groups of bacteria to combat pests. For example, two different sugarcanes-associated bacteria modified to express crystalline (Cry) protein (the active protein from *B. thuringiensis*) or chitinase increased sugarcanes larval mortality to 92% (Downing et al., 2000). Liu et al. (2010) engineered a *Bt* bacterial strain to express two different insecticides, thereby exerting high toxicity against the pests *Anomala corpulenta* and *Holotrichia paralella*. Research on the stability of these and other biotechnologically derived bacterial strains under field conditions is needed before conclusions can be drawn regarding the ecological effects of GM microorganisms.

GM insects can be used to control pests of economic importance (Thomas et al., 2000; Wimmer, 2003). For example, by releasing an excessive number of GM males from which no female offspring will
survive, the populations of insect vectors of human disease and/or agricultural pests can be reduced (Thomas et al., 2000). The environmental risk assessment required for releasing such genetically modified insects can be complex, and so far no genetically engineered insect has been approved for use within the European Union (EU) (Benedict et al., 2010; Reeves et al., 2012). However, transgenic mosquitoes are a potential tool for the control or eradication of insect-vectored diseases. To reduce the risk of malaria, one possible strategy relies on the introduction of malaria-refractory transgenes into wild mosquito populations to limit their capacity to transmit the disease, and a similar approach is being developed for dengue fever (Boete et al., 2014; Marshall, 2010).

3. Discussion

Given the role that GMOs are expected to play in meeting future demands for food and energy (Alfred et al., 2014), knowledge of their sustainable use and environmental impact is crucial (Lau et al., 2014). For some GMOs, such as Bt and HT crops, numerous studies have been published and there is a vast amount of scientific knowledge and practical experience of these organisms. For these crops, direct effects of GM traits are rare and effects on ecosystem processes have seldom been documented. Moreover, many of the published reports on the effects of GMOs are descriptive and lack functional-mechanistic analyses aiming at the causal relationships between organismal traits and relevant ecosystem processes in agro-ecosystems or of importance for natural resource management. The focus of most investigations is on risk assessment at species level without considering an ecosystem perspective. There are also numerous conflicting results on the performance and environmental effects of GM crops, especially with regard to effects on crop yield and impacts on non-target organisms. It has previously been pointed out that this type of data is inconclusive or contradictory and that any discussion on the potential of GM crops needs to take these complex results into account (Gilbert, 2013). Some of the conflicting results reported may be because methods with different levels of resolution and ecological context have been used in different studies. Moreover, there is a lack of large-scale studies (farm and landscape scale, e.g. Squire et al., 2003, 2011) or studies conducted over longer time periods (more than five years), although more and more long-term studies are being published (see for example Lu et al., 2010b; Shi et al., 2013). Greenhouse and laboratory studies are an important first step in biotechnology risk assessments, although it is often not possible to directly apply the results to field situations (Birch et al., 2007; Duan et al., 2010). The short-term studies performed to date mostly show a low impact of GMOs on ecological processes, but the evaluation of the ecological sustainability of GMOs requires the use of long-term experiments investigating complex systems located within an ecological context (Duan et al., 2010; Weih et al., 2008; Zeller et al., 2010). The lack of temporal replicates in many of the reported studies obscures the effects of small changes in the genetic composition of a species, since that does not necessarily mean a small change in the evolutionary development of the species, and hence its effect in the ecosystem. Modelling approaches have been successfully used to predict long-term effects of life history traits and management processes on gene flow of, e.g. oilseed rape (Begg et al., 2006) and sugar beet (Beta vulgaris spp.) (Sester et al., 2012), but model outcomes need to be validated in the real world. When doing so, models will probably be increasingly important at time scales relevant for examining the ecological effects of GMOs. Also, as spatial and temporal scales are extended, study systems become more complex, and clever experimental designs with high statistical power and good replication, to account for high background variability of potentially confounding factors, will be crucial to allow meaningful conclusions. The design of such experiments requires good understanding of molecular biology, physiology and ecology, as well as knowledge of the biology of the target organism and the associated management practices.

Most of the effects of GMOs on ecosystem processes that have been reported to date are indirect, appearing to rely on complex, multi-trophic interactions, and are primarily a consequence of changes in agricultural practices associated with e.g. the use of HT and insect-resistant crops. For these HT and Bt traits, the occurrence of resistant weeds and insect pests follows conventional resistance biology. For example, glyphosate-tolerant weeds are a consequence of the dominant use of one type of herbicide over large areas and over consecutive seasons (Harker et al., 2012). Another significant aspect is the genetic context into which a GM trait is applied. An organisms’ intrinsic biology, such as mode of pollination, is of particular importance, as seen in the example on gene flow from HT oilseed rape and creeping bentgrass. Even within a similar genetic context, the indirect effects of GMOs may differ, as exemplified by reported effects on mycorrhizal fungi. For example, mycorrhizal colonization was reduced in the roots of Bt maize when plants were grown with limited fertilizer applications in a greenhouse (Cheeke et al., 2012b), but there were no differences in mycorrhizal colonization detected between these same Bt maize and non-Bt maize cultivars when grown under high fertilizer conditions (Cheeke et al., 2011) or when cultivated in the field (Cheeke et al., 2013). Thus, the effects of Bt plants on mycorrhizal fungi appear to be context dependent, highlighting the importance of performing studies under varying environmental and experimental conditions.

The knowledge gaps we found highlight an urgent need for basic ecological and agronomic research on the impacts of traits (modified with conventional methods or GM) on ecosystem processes in order to evaluate the possible effects of GMOs in an appropriate setting. Thus, also baseline studies (Conner et al., 2003; Marvier, 2011; Squire et al., 2003) on e.g. above and belowground biodiversity in different types of production systems are required to evaluate the effects of production per se, and to be used as controls for studies of effects of GMOs. With extensive knowledge and understanding of the effects of production and utilisation of natural resources on ecosystem processes, biotechnology can also be used as a powerful tool to gain important insights into how specific traits can affect biodiversity, nutrient cycling, greenhouse gas fluxes and net biomass production in different ecosystems, thereby increasing our general understanding of how single traits affect ecosystem processes. It is well known that epistatic and pleiotropic effects can occur when genes are inserted into an organism, since the insertions can be random. Evaluating how organisms with different insertion sites in the genome perform in different environments may increase our understanding of the effects of genetic and environmental interactions, and enable GMOs to be evaluated in an evolutionary context, i.e. assessing how their fitness is affected by different environments. The study of genetic interactions among genotypes and their abiotic environment in complex communities has been discussed in ecology within the context of community or ecosystem genetics (Whitham et al., 2006). This research usually focuses on the loss of genotypes and the consequences for biodiversity and ecosystem function (Schwartz et al., 2000), but by studying the addition of genotypes to an ecosystem and evaluating them in multiple environments, a better understanding of how species and their functional traits affect ecosystem processes and services can be gained. These types of studies require a clear focus on the traits of GMOs and of conventional organisms and how they are linked to ecosystem processes and services (Constanzo and Barberi, 2014; Weih et al., 2014). Understanding trait effects on ecosystem level processes is even more crucial given the
development of new molecular techniques such as CRISPR/cas which result in biotechnologically enhanced organisms that may or may not be classified as non-GM, but which have traits that can affect ecosystem functioning. The development of advanced technologies resulting in modified traits of organisms not classified as GMOs are, in our opinion, a strong argument to focus future research towards investigating the functional relationships between (modified) traits and ecosystem processes, instead of further evaluating the method (e.g. GM) used to achieve the trait modification. Moreover, understanding the links between traits and ecosystems processes is an important tool in improving cropping systems for efficient resource utilisation with low environmental impact. Studies on GM traits in natural habitats should be incorporated in basic ecological systematic research rather than being a separate research area of risk assessment. By doing so, researchers can utilise the possibility to exploit GM traits to improve the understanding of the relationship between traits and ecosystem processes. Traits will continue to be improved and added to agro-ecosystems, and need to be evaluated irrespective of the technique by which they were produced.

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