

## Environmental Toxicology

## Potential Impact of Effects on Reproductive Attributes Induced by Herbicides on a Plant Community

Jette Reeg,<sup>a,\*</sup> Simon Heine,<sup>b</sup> Christine Mihan,<sup>b</sup> Thomas G. Preuss,<sup>b</sup> Sean McGee,<sup>c</sup> and Florian Jeltsch<sup>a,d</sup><sup>a</sup>Department of Plant Ecology and Nature Conservation, University of Potsdam, Potsdam, Germany<sup>b</sup>Bayer, Monheim am Rhein, Germany<sup>c</sup>Bayer CropScience, Research Triangle Park, North Carolina, USA<sup>d</sup>Berlin-Brandenburg Institute of Advanced Biodiversity Research, Berlin, Germany

**Abstract:** Current herbicide risk assessment guidelines for nontarget terrestrial plants require testing effects on young, vulnerable life stages (i.e., seedling emergence [and subsequent growth] and vegetative vigor [growth and dry wt]) but not directly on the reproduction of plants. However, the European Food Safety Authority (EFSA) has proposed that effects on reproduction might be considered when evaluating the potential effects on plants. We adapted the plant community model for grassland (IBC-grass) to give insight into the current debate on the sensitivity of reproductive versus vegetative endpoints in ecological risk assessment. In an extensive sensitivity analysis of this model, we compared plant attributes potentially affected by herbicides and the consequences for long-term plant population dynamics and plant diversity. This evaluation was implemented by reducing reproductive as well as vegetative endpoints by certain percentages (e.g., 10–90%) as a theoretical assumption. Plant mortality and seed sterility (i.e., inability of seeds to germinate) were the most sensitive attributes. Our results indicated that effects on seed production at off-field exposure rates must be very strong to have an impact on the risk assessment. Otherwise, effects on seed production are compensated for by the soil seed bank. The present study highlights the usefulness of community level modeling studies to support regulators in their decisions on the appropriate risk assessment endpoints and provides confidence in their assessments. *Environ Toxicol Chem* 2018;9999:1–16. © 2018 SETAC

**Keywords:** Ecological risk assessment; Ecotoxicology; Herbicide; Plant reproduction; Plant community model; Nontarget terrestrial plants

## INTRODUCTION

Weeds are undesired plant species in a particular environment. In agricultural fields, weeds are plant species that compete with crop species and often lead to reduced yields of up to 30% (Bagg et al. 2017). To maintain crop health and yields, weeds must be controlled within fields. Various methods may be available for this (e.g., mechanical weeding, thermal or chemical weed control; Bagg et al. 2017), depending on the agricultural practices, labor, and technology available. In conventional intensive agriculture, chemical weed control via herbicides is the most practical and common method applied. With different modes of action, herbicides affect the growth and survival of target plant species. Herbicides may target all

noncrop species in an agricultural field (in-field) or specific monocotyledonous or dicotyledonous plant species. Outside the agricultural field (off-field), in the field boundary and direct neighborhood of an agricultural field, these species are considered nontarget and therefore exposure to herbicides should be limited to conserve semi-natural plant communities. Nevertheless, a combination of certain situations such as weather conditions, application methods, and landscape structure might result in the drift of low doses of herbicides into these off-field areas. According to the Environmental Risk Assessment Scheme, these potentially occurring risks need to be mitigated by buffer zones or drift reduction technology (European and Mediterranean Plant Protection Organization 2003).

To identify these risks, the toxicological sensitivity of plants, that is, the impact of a specific herbicide on individual plants, must be evaluated and conclusions need to be drawn on appropriate application patterns that are predicted to not cause unreasonable harm to off-field nontarget plants. The

This article includes online-only Supplemental Data.

\* Address correspondence to jreeg@uni-potsdam.de

Published online 26 February 2018 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/etc.4122

Organisation for Economic Co-operation and Development (OECD) and the US Environmental Protection Agency (USEPA) have developed standardized greenhouse experiments—required by regulatory authorities worldwide to characterize the toxicological sensitivity of nontarget plants—that can be employed to define herbicide uses safe for nontarget plant populations. In these experiments, the potential effects on plant biomass, shoot length, survival, phytotoxicity, and seedling emergence (vegetative vigor test and seedling emergence test, Organisation for Economic Co-operation and Development 2006a, 2006b; US Environmental Protection Agency 2012a, 2012b, 2012c) are assessed after a herbicide product is applied to the young, vulnerable plants or to the soil surface.

In a deterministic risk assessment scheme, the toxicologically most sensitive endpoint from the seedling emergence and the vegetative vigor tests (i.e., lowest effect rate that was derived for emergence, survival, plant weight, or shoot length) are considered when evaluating the necessity of mitigation measures. If herbicide rates predicted for off-field nontarget areas are lower than the toxicologically most sensitive effect rate value that is combined with an assessment factor to cover uncertainties, it is assumed that the herbicide will not have significant effects on nontarget terrestrial plants, thus protecting off-field plant populations and communities.

The current OECD and USEPA guidelines do not require endpoints that directly measure the reproduction of plants, for example, seed number or fertility of those seeds. However, the European Food Safety Authority (EFSA) recently proposed that effects on reproduction might be considered when evaluating the potential effects on plant populations (European Food Safety Authority, Panel on Plant Protection Products and Their Residues 2014), especially for plant species whose population dynamics rely on seed production. If there is a high seed loss or produced seeds are not fertile, plant populations might decline and eventually become extinct. There are several experimental studies investigating herbicide effects on reproduction and comparing them with vegetative endpoints; however, no general agreement has been reached on a higher or lower toxicological sensitivity of reproductive endpoints, that is, whether the effect rate values for reproductive endpoints are lower than those for vegetative endpoints. Boutin et al. (2014) summarized 3 different ways that herbicides might potentially impact plant reproduction: 1) plant individuals might show a delayed effect on seed production if they are sprayed at earlier stages (e.g., seedling stage), 2) plant individuals at reproduction stage during spray might show a decrease in seed production, or 3) F1 generation might be affected after the mother plant has been sprayed. In an earlier study, Boutin et al. (2000) tested the first 2 pathways and sprayed multiple plant species at different growth stages. When sprayed in the cotyledon and 2 true-leaf stage (this is the guideline-recommended stage for standard vegetative vigor tests), all tested species showed stronger effects on the vegetative vigor than when sprayed at a later stage. Nonetheless, if plants were sprayed during flower bud or at the onset of flowering, effects on reproductive growth and success were more pronounced, supporting the importance of the plants' stage during herbicide application. Other researchers

who compared reproductive endpoints (as a measure of reproductive success) and short- as well as long-term vegetative endpoints concluded that the individual level sensitivity of reproductive endpoints is species- and compound-dependent (Riemens et al. 2008: glufosinate ammonium influenced seed production in *Stellaria media*, but had no effects on seed size if plants are sprayed at an early stage; Riemens et al. 2009: 3 out of 6 plants sprayed with tepraloxydim showed lower sensitivity of reproductive endpoints; Carpenter et al. 2013: 3 out of 5 plants showed effects on long-term reproduction). Overall, these ambiguous results suggest that further research is needed to assess the importance of reproductive endpoints in the framework of registration and regulation of herbicides.

As a further challenge, there is still a lack of knowledge concerning the ecological relevance of individual level endpoints for plant populations and communities over the long term. The European Food Safety Authority (2010) stated that important protection goals are on the level of functional groups, populations, and communities. Although adverse effects may be observed in single individuals in a guideline study, they have the potential to recover over time (as shown in Carpenter and Boutin 2010) or to be compensated by a persistent seed bank or seed dispersal if regarded from a population point of view. Accordingly, it is necessary to increase the knowledge of long-term effects on populations and communities (Barnthouse 2004; Carpenter and Boutin 2010).

Ecological models are well-established tools to analyze long-term dynamics that can often not be investigated in experimental studies (Shugart 1989). In our approach, we adapted the spatially explicit, individual-based plant community model for grasslands (IBC-grass; May et al. 2009; Weiss et al. 2014; Reeg et al. 2017) to compare herbicide-induced effects on vegetative vigor and plant survival with effects on reproduction (seed sterility, establishment, and seed production) and their consequences for long-term plant population dynamics and plant diversity. Because the IBC-grass model is an individual-based model, it is well suited to transfer the individual level effects to plant population and community level. In addition, it applies the trait-based approach, that is, classifying plant species into plant functional types (PFTs) with similar trait characteristics; this is supported by the EFSA because PFTs are suitable to link plant diversity to ecosystem processes (Dáz and Cabido 2001; European Food Safety Authority, Panel on Plant Protection Products and Their Residues 2014).

With this modeling approach, we compare the consequences of herbicide-induced effects on reproductive and vegetative endpoints on long-term population and community dynamics. Furthermore, we aim to give insight into the ecological relevance of the current risk assessment scheme based on vegetative endpoints.

## MATERIALS AND METHODS

### IBC-grass

As noted previously, IBC-grass is a spatially explicit, individual-based plant community model. Main drivers such

as interspecific and intraspecific competition for resources and space as well as disturbances such as grazing, trampling, and mowing are taken into account to reproduce/simulate natural plant community dynamics. The first version of this model was published by May et al. (2009), and was further developed by several researchers (Körner et al. 2014; Weiss et al. 2014). The model version presented in the present study is based on the one described in Reeg et al. (2017). Because of modifications in the above- and below-ground resource levels, the disturbances, and the species pool, the model can be also applied for field boundaries. Additional modifications were performed in the herbicide effect submodel. A detailed model description adhering to the overview, design concepts, and details protocol (Grimm et al. 2006, 2010) can be found in Supplemental Data A. In the following paragraphs, we will provide a short summary of the main principles, processes, and functions of IBC-grass and a detailed explanation of the modified herbicide effect model.

## Main principles

**Trait-based approach.** The concept of functional types is often used to explain ecosystem functioning and dynamics (Gross et al. 2017). Species with similar trait characteristics are expected to respond in a similar way to abiotic and biotic conditions. Therefore, these species can be summarized in one PFT. As a result, general processes and dynamics can be simulated for a specific grassland community, whereas still allowing conclusions for other grassland communities with different species but similar PFT composition. In IBC-grass, important traits and trait syndromes (i.e., a set of correlated traits) for grassland dynamics are selected (see Table 1 for an

overview of the selected traits). Based on the grassland community of interest, species occurring in this community are classified into PFTs according to their trait characteristics. The trait databases BioFlor, LEDA, and cloPla3 are used to collect the specific trait characteristics (Klotz et al. 2002; Kleyer et al. 2008; Klimešová and de Bello 2009). The species composition is based on an extensive literature review by K. Bergholz (University of Potsdam, Potsdam, Germany, unpublished data) on herbaceous field boundaries in Europe (see Table 2 for community characteristics). In this model version we distinguish between monocotyledonous and dicotyledonous PFTs accounting for selective herbicides (see text under *Species-specific susceptibilities* heading in *Materials and Methods* section). The classification into monocotyledonous and dicotyledonous PFTs only relates to herbicide susceptibility and does not involve other trait characteristics. An overview of the plant species and their classification to a specific PFT (with a specific PFT ID) can be found in Supplemental Data B, Table SB1. The PFT ID is composed of the 4 trait syndromes: plant size (small [S], medium [M], and large [L]), growth form (erect [E], semi-rosette [S], and rosette [R]), resource response type (competitor [C], stress-tolerator [S], and intermediate [I]), and grazing response type (tolerator [T], avoider [A], and intermediate [I]); the clonal type (acclonal, short internodes with resource sharing [cl1], short internodes without resource sharing [cl2], long internodes with resource sharing [cl3], and long internodes without resource sharing [cl4]); and cotyledon characteristic (monocotyledonous [m] and dicotyledonous [d]). For each PFT group, we give an example of a plant species belonging to that group. The characteristics of each PFT ID and the associated plant species can be found in Supplemental Data B, Table SB1.

**TABLE 1:** Trait syndromes, plant functional type-specific trait parameter values, and databases used

Trait/trait syndrome and attributes	Trait parameters				Database
Growth form	$f_{\text{leaf}}$				BioFlor—rosette attribute,
Rosette	1.0				rosette,
Intermediate	0.75				semi-rosette,
Erect	0.5				erect
Maximum plant size	$m_{\text{max}}$	$m_{\text{seed}}$	$\text{mean}_{\text{disp}}$	$\text{std}_{\text{disp}}$	LEDA—seed-releasing height
Large	5000 mg	1 mg	0.1 m	0.1 m	> 0.87 m
Medium	2000 mg	0.3 mg	0.3 m	0.3 m	0.42–0.87 m
Small	1000 mg	0.1 mg	0.6 m	0.6 m	< 0.42 m
Resource response	$r_{\text{Umax}}$	$\text{surv}_{\text{max}}$			BioFlor—plant strategy type
Competitor	60 resource units	2 wk			c, cr
Intermediate	40 resource units	4 wk			csr, r
Stress-tolerator	20 resource units	6 wk			sr, cs, s
Grazing response	Palat	$C_{\text{shoot}}$			BioFlor—indicator values grazing tolerance
Tolerator	1.0	1.0			4–6
Intermediate	0.5	0.75			1–3
Avoider	0.25	0.5			7–9
Herbicide susceptibility	Sens 0–1				Randomly distributed
Clonal integration	Resshare				cloPla3—persistence of connection
Integrator	1				≥ 2 yr
Splitter	0				< 2 yr
Lateral spread	SpacerL	$\text{std}_{\text{SpacerL}}$			cloPla3—lateral spread
Short	2.5 cm	2.5 cm			< 0.01 mo/yr
Long	17.5 cm	12.5 cm			0.01–0.25 mo/yr and longer

Palat = palatability; Sens = sensitivity; Resshare = resource sharing between spacer.

**TABLE 2:** Summary of abiotic parameters of the simulated plant community<sup>a</sup>

	Field-boundary community
Below-ground resources	High
Disturbances	
Cutting per year (at 500 mg dry wt/cm <sup>2</sup> )	1
Trampling (% area/year)	10%

<sup>a</sup>The community is based on a literature review by Kolja Bergholz (K. Bergholz, University of Potsdam, Potsdam, Germany, unpublished data).

**2-layer zone-of-influence approach.** The IBC-grass simulates competition between plant individuals via the zone-of-influence approach—distinguishing between above ground and below ground. Individuals are assumed to acquire resources in an area around their location—their zone of influence. If zones of influence of neighboring individuals overlap, they compete for resources (and space). In IBC-grass, the zone of influence is modeled as a circular area around the stem of an individual. Above ground, the size of the area is determined by the shoot geometry (e.g., rosette) and the shoot mass/size. Above ground the plants compete for light. Competition is simulated partially size-asymmetrically in the way that taller plants acquire more light resources proportional to their shoot mass and geometry. Below ground, it is assumed that the roots of the plants have similar growth forms. Therefore, the below-ground zone of influence depends only on the root mass, that is, high root mass results in a larger zone of influence. In this compartment, competition for resources in overlapping zones of influence is simulated size-symmetrically (i.e., competition is independent of the root size/mass and actual zone of influence).

**Spatial dimensions and main state variables.** Community dynamics are simulated on a local patch of approximately 3 m<sup>2</sup>. This patch is divided into smaller grid cells of 173 × 173 cells of 1 cm<sup>2</sup> each. Each grid cell can contain the stem of 1 plant individual and several seeds. The main state variables are the location (x coordinate, y coordinate), the current shoot, root, and reproductive mass, and the resulting zone of influence of a plant individual, and the location x coordinate and y coordinate of a seed. Periodic boundaries are simulated to avoid edge effects. The local patch is similar to a torus, that is, the edges to the left and right as well as upper and lower edges are connected.

**Main processes.** The schedule of the main processes is presented in a flowchart (Figure 1). In the following paragraphs, we will give a short summary of these processes including the main equations. For a detailed explanation see Supplemental Data A.

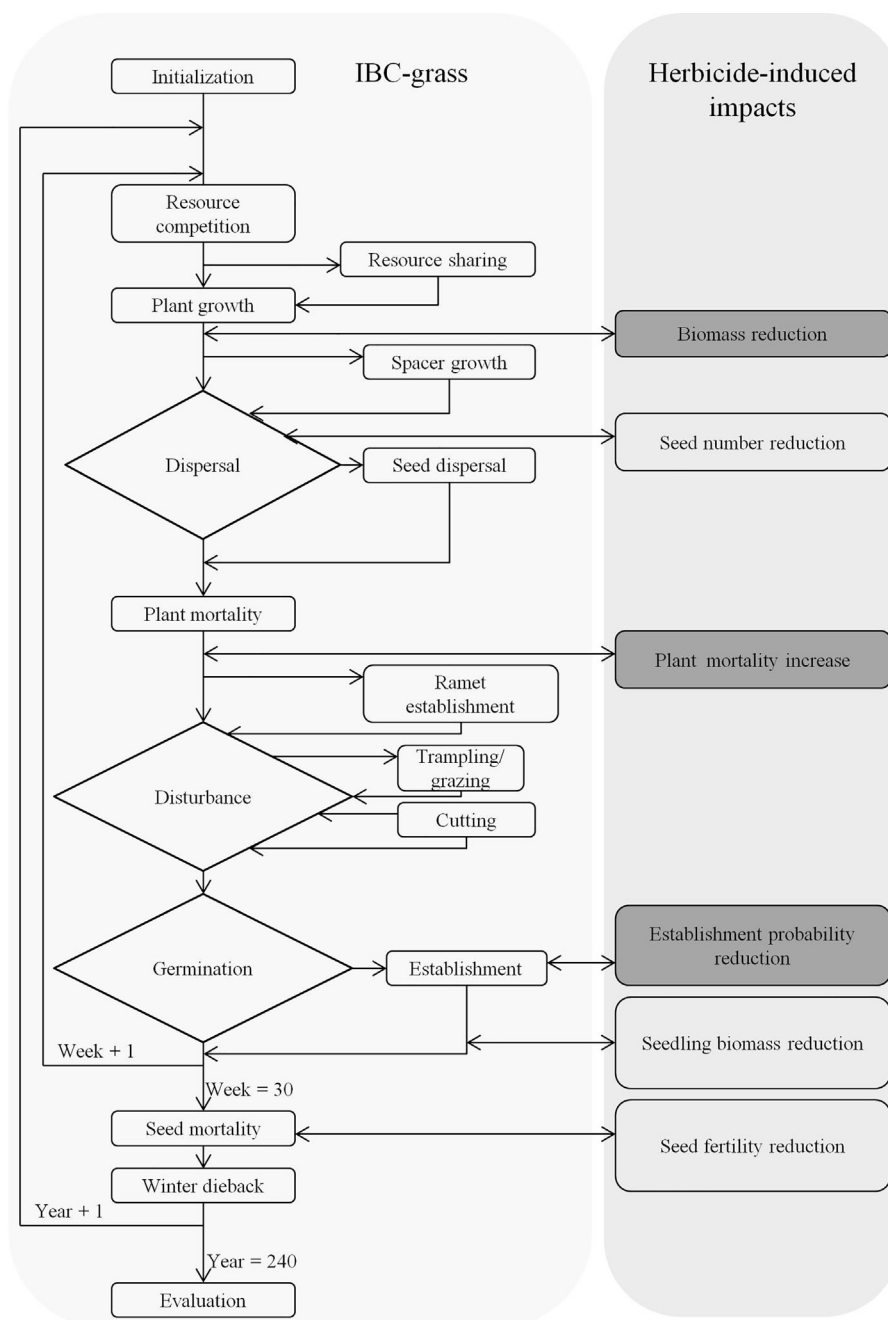
**Interactions and growth.** Plant individuals acquire resources,  $\Delta_{res}$ , in their zones of influence. In areas of overlapping zones of influence they compete for resources (see paragraph introduced by 2-layer zone-of-influence approach in Materials and Methods section), with intraspecific competition being stronger than interspecific competition (for details see the overview, design

concepts, and details protocol, Supplemental Data A). The acquired resources are allocated to roots and shoots and converted into biomass based on the constant conversion rate  $g$ , the current shoot (root) mass  $m_{shoot(root)}$ , the trait characteristics of the shoot (root)  $c_{shoot}$  (RAR), the growth form  $f_{leaf}$ , the maximal resource utilization  $ru_{max}$ , and the maximal plant mass  $m_{max}$ . In the absence of competition, this growth function results in a sigmoid growth (Table 3, shoot/root growth Equation; see also DeAngelis and Mooij 2005; Berger et al. 2008). Clonal PFTs invest 5% of their acquired resources in the growth of one spacer (rhizome or stolon connecting ramets of clonal plants) per time. The direction of the growing spacer is drawn randomly from a uniform distribution and the distance until establishment is randomly chosen from a normal distribution based on the PFT-specific mean spacer distance (SpacerL; Table 1). If the spacer reaches its maximal distance, spacer growth stops and the new ramet establishes with a constant probability  $p_{ram}$  of 50% if the current cell is not the location of the stem of another plant individual. If the current cell is occupied, the spacer can continue to grow within a radius of 2 cells. If the ramet is established, a new spacer is developed. Therefore, the lateral growth of clonal PFTs is not limited.

**Mortality.** Plant individuals can die as a result of resource stress, random density-dependent mortality, and winter dieback. The probability of death caused by resource stress increases with the duration of resource stress and depends on the characteristics of the trait  $w_{stress}$ , which is the maximal number of consecutive weeks of resource stress the plant is able to withstand; that is, the closer the plant individuals get to their maximum survival time under resource stress (i.e., lack of resources to maintain base metabolism), the higher their mortality probability. The basic background mortality of 0.7% corresponds to an annual mortality of 20% (Schipper et al. 2001). It escalates exponentially with increasing population size of the specific PFT to account for density-dependent impacts (e.g., diseases and pests; Reynolds et al. 2003; Bell et al. 2006). During winter, above-ground biomass is decreased by 50% to account for winter dieback. If the resulting biomass is below 10 mg, the plant is considered dead (May et al. 2009; Körner et al. 2014; Weiss et al. 2014). Seeds suffer from winter mortality (50% mortality probability during winter) and die because of age (maximal age = 1; i.e., there is no long-term seed bank simulated).

**Seed dispersal, germination, and establishment.** During the seed production period, plant individuals invest 5% of their acquired resources in the production of seeds (Schipper et al. 2001). In the following week of this period, all plants disperse their seeds. The direction is drawn from a uniform distribution and the dispersal distance depends on the PFT-specific seed characteristics and is drawn from a log-normal distribution (Stoyan and Wagner 2001).

During the 2 seed establishment periods in the beginning of each simulated year (spring) and after seed dispersal (summer), seeds germinate with a probability of 50% (May et al. 2009; Körner et al. 2014; Weiss et al. 2014) and establish on bare



**FIGURE 1:** Overview and chronology of the main processes in individual-based plant community model for grasslands (IBC-grass) including herbicide-induced impacts. Dark gray boxes mark plant attributes currently tested in ecotoxicological standard tests.

ground (i.e., on cells that are not covered by any other individual). The chance of successful establishment depends on the seed mass, with heavier seeds assumed to be more competitive.

**Abiotic factors.** In IBC-grass, resources (above- as well as below-ground) are distributed homogeneously in space and time. In addition, biotic disturbances such as grazing (selective removal of above-ground biomass), trampling (removal of individuals), and cutting (removal of above-ground biomass down to a certain height) are included. See the overview, design

concepts, and details protocol in Supplemental Data A for detailed information.

**Temporal dimensions.** IBC-grass simulates the vegetation period of 30 wk per year (starting in spring, ending at the beginning of winter). As a result of environmental filtering and competition among PFTs, the PFT community is within a stable state after 150 yr. To avoid population and community level effects caused by this stabilization process, simulated herbicide application starts after another 25 yr (in year 175) and ends after 30 yr.



**TABLE 3:** Main equations in the individual-based plant community model for grasslands regarding competition, growth, and mortality<sup>a</sup>

Process	Equation	Variables
Zone of influence	$A_{\text{shoot}} = c_{\text{shoot}} \times (f_{\text{leaf}} \times m_{\text{shoot}})^{2/3}$	$c_{\text{shoot}}$ —PFT-specific leaf area $RAR$ —root form (1)
Resource competition	$A_{\text{root}} = RAR \times m_{\text{root}}^{2/3}$ $\Delta \text{res}_i = \frac{\beta_i}{\sum_{j=1}^n \beta_j} \times \text{Res}_{\text{cell}}$	$f_{\text{leaf}}$ —PFT-specific growth form $m_{\text{shoot/root}}$ —shoot/root mass (mg dry wt) $\text{Res}_{\text{cell}}$ —resources in cell
Asymmetric competition	$\beta_i = r_{\text{u}_{\text{max}}} \times m_{\text{shoot}} \times f_{\text{leaf}}^{-1}$	$r_{\text{u}_{\text{max}}}$ —PFT-specific maximal resource utilization
Symmetric competition	$\beta_i = r_{\text{u}_{\text{max}}} \times \frac{1}{\sqrt{n_{\text{PFT}}}}$	$g$ —conversion rate resources to biomass $n_{\text{PFT}}$ —number of neighboring PFTs
Shoot growth	$\Delta m = g \times (\Delta \text{res} - c_{\text{shoot}} \times f_{\text{leaf}}^{2/3} \times r_{\text{u}_{\text{max}}} \times \frac{m_{\text{shoot}}^2}{m_{\text{max}}^{4/3}})$	$m_{\text{max}}$ —PFT-specific maximal plant mass (mg dry wt) $w_{\text{stress}}$ —consecutive weeks under stress
Root growth	$\Delta m = g \times (\Delta \text{res} - RAR \times r_{\text{u}_{\text{max}}} \times \frac{m_{\text{root}}^2}{m_{\text{max}}^{4/3}})$	$\text{surv}_{\text{max}}$ —PFT-specific maximal survival under stress (weeks)
Density-dependent mortality	$P_{\text{mort}} = P_{\text{base\_new}} + \frac{w_{\text{stress}}}{\text{surv}_{\text{max}}}$ with $P_{\text{base\_new}} = P_{\text{base}} \times \left(1 + e^{\frac{5 \times \text{current\_abundance}}{\text{max\_abundance}}}\right)$	$p_{\text{base}}$ —base mortality (0.7%) current_abundance—current abundance of a PFT max_abundance—maximal potential abundance of a PFT (assuming fully grown individuals)

<sup>a</sup>More details can be found in the *Materials and Methods* section and the ODD protocol in Supplemental Data A.

ODD = overview, design concepts, and details protocol; PFT = plant functional type.

## Implementation of herbicide-induced impacts

Herbicides potentially have an effect on the following plant attributes (according to the Organisation for Economic Co-operation and Development 2006a, 2006b; the US Environmental Protection Agency 2012a, 2012b, 2012c; Boutin et al. 2014): 1) Survival of plants: Implementation in IBC-grass: plants suffer from an additional herbicide-induced mortality probability. The strength of effect is determined by the specific effect intensity and is added after the density-dependent mortality. 2) Biomass of seedlings and plants: Implementation in IBC-grass: the biomass of seedlings and biomass gain of plants is reduced according to the specific effect intensity. 3) Establishment (emergence) of seedlings: Implementation in the IBC-grass model: the establishment probability of seedlings is reduced by the specific effect intensity. 4) Produced seed number: Implementation in the IBC-grass model: the resources available for seed production are reduced by the specific effect intensity. The PFT-specific seed weight is kept constant, resulting in a lower number of produced seeds. Finally, 5) Sterility of seeds: Implementation in IBC-grass: seed sterility is increased by adding an herbicide-induced seed mortality probability according to the specific effect intensity that is comparable with seed fertility.

Herbicide application is simulated in the present theoretical case study by an arbitrarily assigned reduction of the plant parameters mentioned earlier (varying from 10–90% effect intensities). No underlying exposure assumptions of a specific herbicide application (e.g., overspray, drift values, etc.) are considered. Herbicide application is simulated in the first week of each simulated year, bearing in mind that only the growing season starting in spring is simulated in IBC-grass (i.e., herbicide application takes place during spring). However, each of the herbicide-induced effects becomes effective only within the first week of the specific process. For example, seed production is simulated only in week 25; therefore, the herbicide effect on seed production occurs within this week 25. In this way, we were able to account for direct effects on each specific attribute. In

addition, not only directly sprayed plants and seed individuals may suffer from an herbicidal effect (parental generation). Recently, concerns have been raised that sprayed plants might also pass on these effects to their offspring/seeds, resulting in effects on the F1 generation (generation carryover effects; Boutin et al. 2014). Consequently, to address this concern in a conservative manner, we accounted not only for different attributes but also included generation carryover effects. The timing of effects and the potentially affected individuals are summarized in Table 4.

## Species-specific susceptibilities

To account for different species-specific herbicide susceptibilities, we tested dissimilar affected PFT groups. 1) All: All PFTs within the community are affected. This accounts for a broad spectrum herbicide ( $n = 55$ ). 2) Competitive: Only competitive PFTs that occur frequently (in more than 80% of the control Monte Carlo runs) in an isolated community are affected. This scenario was chosen to analyze the ecological sensitivity of the community and does not represent a realistic scenario. (Selectiveness of herbicides is mostly based on other trait characteristics of plants such as monocotyledonous versus dicotyledonous species) ( $n = 4$ ). 3) Less competitive: Only less competitive PFTs that occur frequently (in more than 80% of the control Monte Carlo runs) in an isolated community are affected. This scenario was used to analyze the ecological sensitivity of the community as a comparison with the competitive PFTs' scenario and probably does not represent a realistic case ( $n = 4$ ). 4) Monocotyledonous: Only monocotyledonous PFTs are affected. This scenario considers a selective herbicide acting only on monocotyledonous PFTs ( $n = 14$ ). 5) Dicotyledonous: Only dicotyledonous PFTs are affected. This scenario takes into account a selective herbicide acting only on dicotyledonous PFTs ( $n = 40$ ). The PFTs within these groups have the same susceptibility (1). Hence the effect intensity is equal for each of the affected PFTs. The distinction between monocotyledonous

**TABLE 4:** Overview of the timing and affected individuals for each attribute and affected generation

Attribute		Parental generation (P) (excludes generation carryover effects)	Parental + F1 generation (P + F1) (includes generation carryover effects)
Mortality	Week of effect Affected individuals	1 Existing plants in week 1	1–3, 21–25 Existing plants in week 1 + F1 seedlings after their establishment in weeks 1–3 and 21–25
Biomass	Week of effect Affected individuals	4 Existing plants in week 1	4 Existing plants in week 4
Establishment	Week of effect Affected individuals	Weeks 1–3, 21–25 Existing seeds in week 1	Weeks 1–3, 21–25 Existing seeds in week 1 + produced seeds in week 19
Seed number	Week of effect Affected individuals	Week 19 Existing plants in week 1	Week 19 All existing plants
Seed sterility	Week of effect Affected individuals	Week 1 Existing seeds in week 1	Weeks 1 or 20 Existing seeds in weeks 1 + 20

and dicotyledonous PFTs involves only the herbicide susceptibility (affected yes/no) and no other trait characteristics.

## Scenarios

In addition to the different affected attributes (mortality, biomass, establishment, seed number, and seed sterility and the combination of mortality + biomass, mortality + biomass + seed number, and establishment + seed number + seed sterility), the generation (parental and parental + F1 [P and P + F1]), and the PFT group (all, competitive, less competitive, monocotyledonous, dicotyledonous), we also included 2 diverse seed input scenarios (isolated community without seed input [0 seeds/PFT/yr] and a community with moderate seed input [10 seeds/PFT/yr] and different effect intensities [from 10–90% effect intensity]).

## Analyses

The output of the IBC-grass model is the population size per PFT per time step. Based on the population size, the inverse Simpson Diversity Index ( $1/\sum p_i^2$  with  $p_i$  being the proportion of individuals belonging to species  $i$ ) is calculated for each time step. The inverse Simpson Diversity Index was selected for analysis because it showed the strongest impacts in our scenarios compared with other diversity indices such as the Shannon-Wiener Index or evenness.

Each scenario includes 50 Monte Carlo runs (repeated 50 times) because of stochasticity and compared with control scenarios (also 50 Monte Carlo runs) in which no herbicide application is simulated. Each single Monte Carlo run is done in

relation to the mean of the control (i.e., dividing by the mean of the control for both the population size and inverse Simpson Diversity Index). In a next step, the relative means for the treatment simulations and the 2.5th and 97.5th percentiles of the control simulations are calculated and again summarized over the entire herbicide application period of 30 yr (as a mean over 30 yr). If the treatment mean is outside the range of the means for the 2.5th and 97.5th percentiles of the control, a significant effect is assumed. Nevertheless, this type of significance should not be confused with statistical significance.

In this context, ecological sensitivity of a PFT community is the minimal effect intensity (see Table 5 for a definition) that is required to cause significant effects on the population size of 50% of the frequent (frequency > 0.8) PFTs or the minimal effect intensity that causes a significant effect of at least or more than 50% on the inverse Simpson Diversity Index. Ecological sensitivity of a PFT population level is the minimal effect intensity resulting in a mean significant effect extent. Smaller values show that the effect intensity that is applied to a specific PFT trait on the individual level to achieve significant effects is also small. Accordingly, smaller values represent a higher ecological sensitivity and thus a stronger impact on the PFT community and population.

If the analysis detects a significant effect, the mean significant effect was calculated (see Table 5 as an overview of study-specific terms). This measure is the difference between the mean over the 30 yr of simulated herbicide application and the mean lower (or upper, depending on the effect direction [negative or positive]) percentile of the control. If the mean lower percentile (2.5th percentile) in the control is, for example, 0.7 (with 1.0 being the baseline) and the mean relative population size over

**TABLE 5:** Overview and explanation of study-specific terms

Term	Explanation
Effect intensity	Probability or rate of the individual level effect
Significant <sup>a</sup> effect intensity	Effect intensity that leads to a significant effect extent
Significant effect extent	Mean (relative) effect occurring at the population or community level (significant <sup>a</sup> ; if it is outside of the relative 2.5th and 97.5th control percentiles)
Mean significant effect	Difference between the mean control percentiles (2.5th and 97.5th) and the mean significant <sup>a</sup> effect extent

<sup>a</sup>The term “significant” in this context of this table does not refer to the concept of statistical significance.

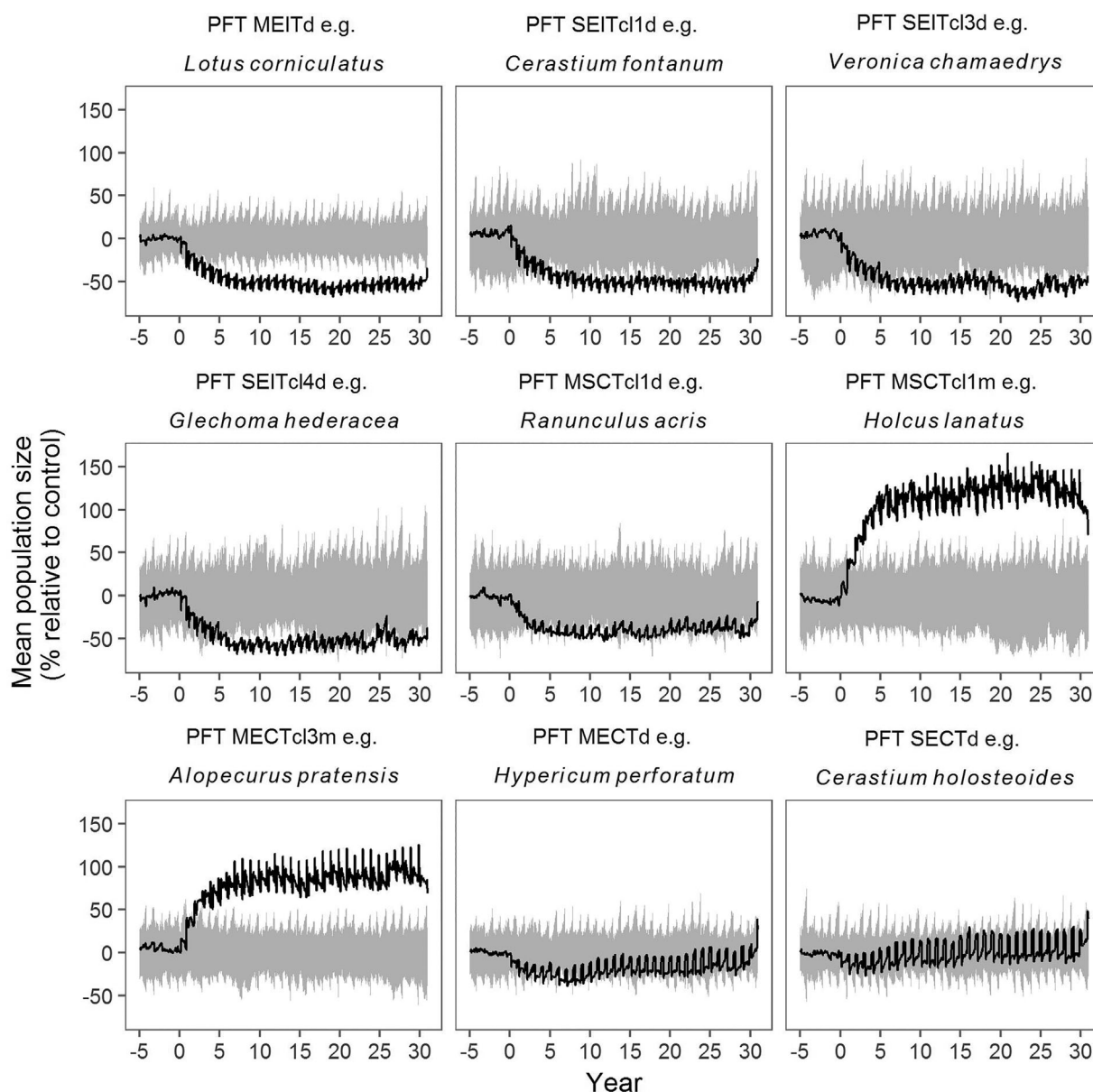
this period in the treatment is 0.5, the mean significant effect would be  $-0.2$  ( $-20\%$ ) but not  $-0.5$  ( $-50\%$ ). In this way, the measure accounts for potential high variation within PFT population sizes in the control.

## RESULTS

### Temporal behavior of the community

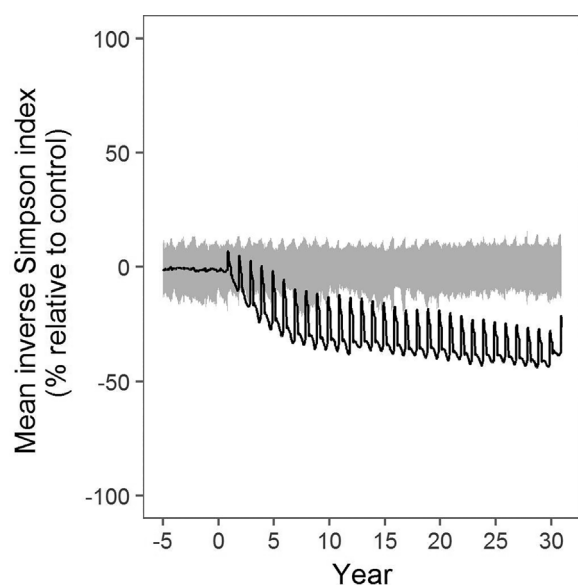
The temporal behavior of the simulated community is shown in Figures 2 and 3. In this exemplary scenario, the attributes seed

number, seed sterility, and establishment are affected by 30% for the parental as well as the succeeding F1 generation (simulating a generation carryover effect) in an isolated community (external seed input = 0). Only dicotyledonous PFTs ( $n = 41$ ) are affected. Frequent dicotyledonous PFTs, marked with a "d" in Figure 2, show a strong decrease in population size. Nonetheless, the extent and temporal response are diverse. The PFTs MEITd (explanation of the PFT IDs can be found in the Materials and Methods section), representing (all plant species listed are examples) *Lotus corniculatus*, and SEIT, representing *Cerastium fontanum*, *Veronica chamaedrys*, and *Glechoma hederacea*, are strongly affected



**FIGURE 2:** Effects of 30% effect intensity (per year) on seed number, seed sterility, and seed establishment on the population size of frequent plant functional types (PFTs) within an isolated community. Only dicotyledonous PFTs (PFT\*d) are affected. Black lines represent the mean in treatment relative to the mean of the control; gray ribbons show the variation within the control (in relation to the control mean). 5 yr before herbicide application (starting in year 0) and 30 yr of simulated herbicide application are displayed. The PFT ID is composed of the 4 trait syndromes: plant size (small [S], medium [M], and large [L]), growth form (erect [E], semi-rosette [S], and rosette [R]), resource response type (competitor [C], stress-tolerator [S], and intermediate [I]), and grazing response type (tolerator [T], avoider [A], and intermediate [I]); the clonal type (acclonal, short internodes with resource sharing [cl1], short internodes without resource sharing [cl2], long internodes with resource sharing [cl3], and long internodes without resource sharing [cl4]); and cotyledon characteristic (monocotyledonous [m] and dicotyledonous [d]).





**FIGURE 3:** Effects of 30% effect intensity (per year) on seed number, seed sterility, and seed establishment on the inverse Simpson Diversity Index within an isolated community. Only dicots are affected. Black line indicates the mean in treatment relative to the mean of the control; gray ribbon identifies the variation within the control (in relation to the control mean). 5 yr before herbicide application (starting in year 0) and 30 yr of simulated herbicide application are shown.

and show a long-lasting impact on population size over the simulated period. The PFT MSCTcl1d, representing *Ranunculus acris*, displays a lower but still long-lasting effect. In contrast, the PFTs MECTd (*Hypericum perforatum*) and SECTd (*Cerastium holosteoides*) indicate only short-term, negative effects. Both PFT populations are able to recover within a year and even reveal short-term increases in population size. The 2 frequent monocotyledonous PFTs MSCTcl1m (*Holcus lanatus*) and MECTcl3m (*Alopecurus pratensis*) display a strong, long-lasting increase in population size with high fluctuations within a year. Differences in the response of PFTs can be explained by the dissimilar trait characteristics (see Reeg et al. 2017 for a detailed discussion).

Looking at the entire community and diversity, a continuous decline in the inverse Simpson Diversity Index can be observed with short-term recovery during the second establishment period in autumn (Figure 3).

### Influence of the generation, the degree of isolation, the PFT group, and the affected attribute

**Community level.** In general, on a community level the inverse Simpson Diversity Index shows a strong decrease when both the parental and the F1 generation are affected (each attribute affected by 50%; Figure 4). An isolated (seed input = 0) community indicates stronger effects than a community with external seed input (seed input = 10). In the scenario where all PFTs are affected at the same intensity, the community displays the lowest negative response in the diversity index. Simulating herbicide-induced impacts which only act on frequent competitive PFTs ( $n = 4$ ), less competitive PFTs ( $n = 4$ ), or dicotyledonous

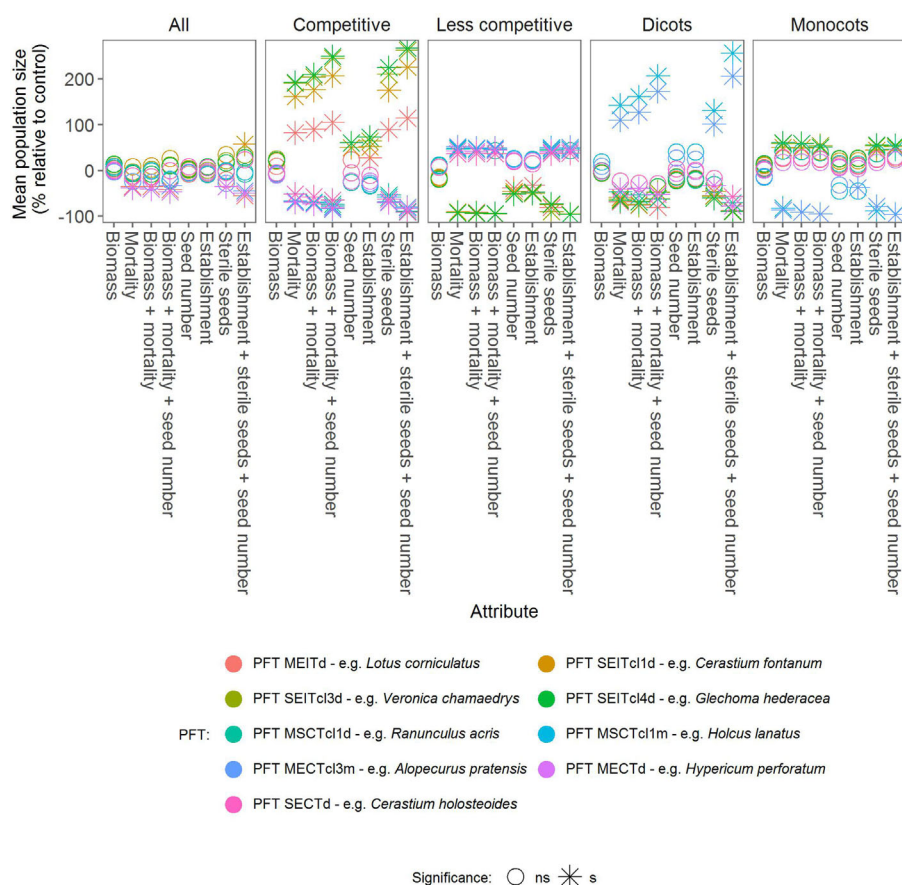
PFTs ( $n = 40$ ) lead to strong negative effects on the diversity index. To summarize, strongest effects are found in an isolated community (seed input = 0), when herbicide-induced impacts target only dicotyledonous species and also affect the F1 generation (P + F1).

Viewing the impacts on a community level for the single affected attributes, an increase in plants' mortality and in seed sterility reveal the strongest effect extents. A combination of affected attributes leads to stronger impacts on the inverse Simpson Diversity Index. The single effects of each attribute do not total in a linear fashion. For instance, in the worst-case scenario—where the parental and F1 generation of dicotyledonous PFTs are affected in an isolated community—a 50% effect intensity on the individual level only on biomass, or mortality, or seed number leads to a 2, 44, or 4% reduction of the inverse Simpson Diversity Index, respectively. A combination of these effects, that is, a 50% effect intensity on the individual level on each of these attributes at the same time, leads to a 61% decrease (slightly larger than the additive effects if only the single attributes are affected [ $2 + 44 + 4\%$ , as mentioned above]). However, when only monocotyledonous PFTs are affected, a 50% effect on the single attributes already noted (biomass, mortality, and seed number) leads to 0, 17, and 4%, respectively. A combination on the other hand results in an even lower effect of only 16%.

**Population level.** Now we focus on the scenario with the strongest effects (P + F1 generations are affected in an isolated community; seed input = 0) as a very conservative scenario combining all the worst-case assumptions.

At the population level, direct negative as well as indirect positive effects are found (Figure 5). When all PFTs are affected by a 50% effect intensity, there are only a few PFTs displaying significant negative impacts on population size (all plant species listed are examples; for the single attributes: PFTs MEITd [*Lotus corniculatus*] and MECTd [*Hypericum perforatum*]; for the combined attributes: also PFT MECTcl3m [*Alopecurus pratensis*]). Only when the 3 reproductive attributes—establishment, seed sterility, and seed number—are affected in combination, PFT SEITcl1d (*Cerastium fontanum*) indicates a significant increase in population size. When only the frequent competitive PFTs are affected, a strong reduction in population size can be observed for these PFTs. Notwithstanding, there is also an indirect effect on the less competitive PFTs (assumed to be insensitive to the herbicide in this scenario), resulting in a strong enhancement of population sizes, especially when the attributes mortality and seed sterility are affected. The divergence between increase and decrease of population sizes is less pronounced when only the frequent less competitive PFTs are affected. However, the tendency is similar: population sizes of affected PFTs are decreased as a direct herbicide-induced effect and unaffected (insensitive) PFTs increase as an indirect effect. Thereby, the extent of the reduction and growth in population sizes is similar between PFTs of the same group (competitive/less competitive). When all dicotyledonous PFTs are affected, their population sizes decrease to different extents depending on the PFT. The 2 monocotyledonous PFTs strongly increase in population size (as an indirect effect). Because there are only 2





**FIGURE 5:** Effects of 50% reduction (effect intensity) in each attribute on the population sizes of all frequent plant functional types (PFTs) within an isolated community (seed input = 0), whereas inheritance is included (P + F1). This theoretical worst-case scenario showed strongest effects on the community level. Significant effects are marked with asterisks (mean of treatment is outside the range of control). The PFT ID is composed of the 4 trait syndromes: plant size (small [S], medium [M], and large [L]), growth form (erect [E], semi-rosette [S], and rosette [R]), resource response type (competitor [C], stress-tolerator [S], and intermediate [I]), and grazing response type (tolerator [T], avoider [A], and intermediate [I]); the clonal type (aclonal, short internodes with resource sharing [cl1], short internodes without resource sharing [cl2], long internodes with resource sharing [cl3], and long internodes without resource sharing [cl4]); and cotyledon characteristic (monocotyledonous [m] and dicotyledonous [d]).

establishment, seed sterility, and seed number). Thus the highest impacts on the PFT community occur when only dicotyledonous PFTs are targeted.

Furthermore, we were also interested in the effect intensities at which the population size and the diversity index showed significant impacts (Figure 7). In this context, ecological sensitivity of a PFT population is the effect intensity at which significant impacts occur—the lower the value the higher the sensitivity. To illustrate, we selected a conservative but still realistic scenario: the parental and the F1 generations are affected, the local community has a moderate seed input (10 seeds/yr/PFT), and the herbicide targets all, dicotyledonous, or monocotyledonous PFTs.

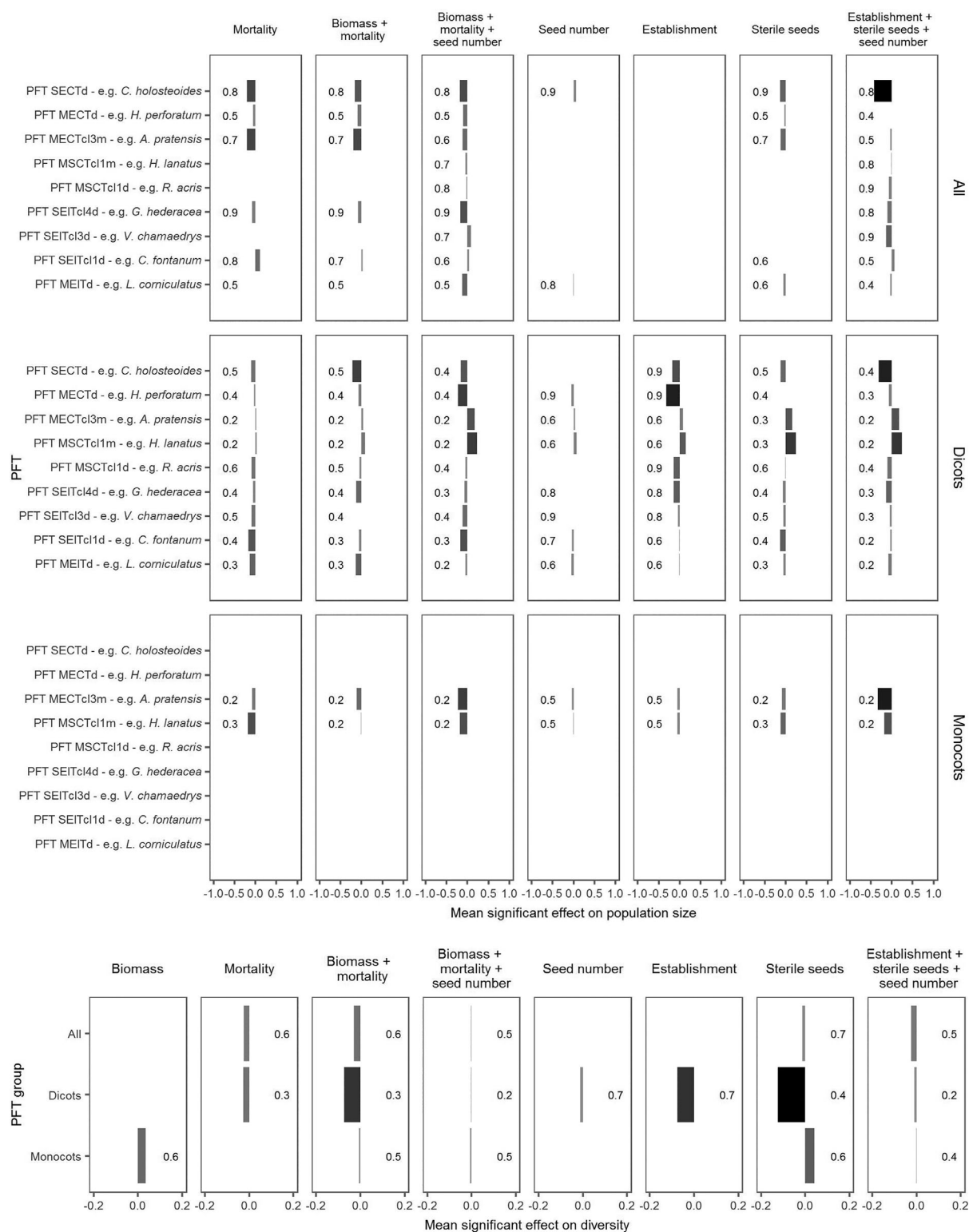
When all PFTs are targeted, the PFTs MECTd (all plant species referred to are examples; *Hypericum perforatum*) and MEITd (*Lotus corniculatus*) are ecologically most sensitive; significant effect extents occur at the lowest effect intensities compared with the other PFT populations, when the combination of reproductive attributes (establishment, seed sterility, and seed number) is affected. An effect intensity of 40% on each attribute leads to the first significant effect extents for these PFTs. However, the mean significant effects (% decrease or increase below or above the 2.5th or 97.5th percentile of the control; see *Materials and*

*Methods* section for more information) are only a 4% (PFT MEITd, *Lotus corniculatus*) and a 0.2% (PFT MECTd, *Hypericum perforatum*) decrease. For this attribute combination, the population sizes of the other PFTs indicate a significant effect only at 50 to 90% effect intensity with a mean significant effect of –42% (decrease) through 7% (increase). For the other 2 attribute combinations (biomass and mortality; biomass, mortality, and seed number) and the single attribute of mortality, the lowest significant effect intensity was between 50 to 90% with mean significant effect extents of –21% (decrease) through 11% (increase). The PFTs SECTd (*Cerastium holosteoides*), MECTd (*Hypericum perforatum*), MECTcl3m (*Alopecurus pratensis*), SEITcl1 (*Cerastium fontanum*), and MEITd (*Lotus corniculatus*) display significant impacts on population level at an effect intensity of 50 to 90% on the attribute of seed sterility with negative mean significant effects of up to 13%. For all PFTs, an effect on the seed number and the establishment does not have significant impacts up to an effect intensity of 80%.

When only dicotyledonous PFTs are affected, population sizes of both monocotyledonous PFTs MECTcl3m (*Alopecurus pratensis*) and MSCTcl1m (*Holcus lanatus*) increase significantly when the single attribute of mortality or the attribute







**FIGURE 7:** Mean significant effect extent on each plant functional type (PFT) population (3 top rows, effects on population size) and the community (bottom row, effects on inverse Simpson Diversity Index). Numbers show the minimal effect intensity resulting in a mean significant effect extent illustrated by the height of the bars. The lower the numbers, the higher the sensitivity of the PFT. The image indicates only the more realistic scenarios with seed input (10 seeds), where both generations P and F1 are affected (inheritance is included). The PFT ID is composed of the 4 trait syndromes: plant size (small [S], medium [M], and large [L]), growth form (erect [E], semi-rosette [S], and rosette [R]), resource response type (competitor [C], stress-tolerator [S], and intermediate [I]), and grazing response type (tolerator [T], avoider [A], and intermediate [I]); the clonal type (acclonal, short internodes with resource sharing [cl1], short internodes without resource sharing [cl2], long internodes with resource sharing [cl3], and long internodes without resource sharing [cl4]); and cotyledon characteristic (monocotyledonous [m] and dicotyledonous [d]).



seed number and establishment indicate low ecological sensitivities only at effect intensities of 60 to 90%.

When monocotyledonous PFTs are targeted, there are no significant (indirect) effects for dicotyledonous PFTs. However, the 2 frequent monocotyledonous PFTs are quite sensitive to effects on all single attributes and attribute combinations (i.e., there are significant effects at effect intensities of 20–30%), except for the single attributes of seed number and establishment (significant effects at effect intensities of 50%).

The inverse Simpson Diversity Index is ecologically most sensitive in the scenario where dicotyledonous PFTs are targeted; in other words, there is a high impact on the diversity already at low effect intensities. Especially if a 3-fold combination of affected attributes is considered, the inverse Simpson Diversity Index shows strong impacts. At effect intensities of 20% on each of the attributes, the index shows a mean significant decrease of 0.2% (biomass, mortality, and seed number) through 2% (establishment, seed sterility, and seed number).

## DISCUSSION

Reproductive performance is an important factor for plant population and community dynamics. However, the toxicological sensitivity of plants to reproductive impairment is not well characterized and current standard ecotoxicological tests (Organisation for Economic Co-operation and Development 2006a, 2006b; US Environmental Protection Agency 2012a, 2012c) do not directly address reproductive endpoints such as seed production. To address the uncertainty associated with reproductive endpoint sensitivity in the ecotoxicological tests, we adapted the individual-based plant community model for grasslands (IBC-grass) to compare the consequences of herbicide-induced effects on vegetative endpoints (biomass and plant mortality) with effects on reproductive endpoints (seed sterility, seed establishment, and seed production) at the population and community level. Because we were interested in a general understanding of potential differences between vegetative and reproductive endpoints, we systematically varied herbicide effects by a variable, randomly chosen percentage of reduction of plant biomass or survival in comparison with reduced reproduction parameters rather than focusing on specific exposure patterns related to actual herbicidal products.

Seed sterility (ability of seeds to germinate) and plant mortality were the ecologically most sensitive attributes in the present study, showing significant impacts on population and community levels already at low effect intensities. An herbicide-induced increase in seed sterility or plant mortality even at low effect intensities (individual level effect on the specific attribute) resulted in the highest impacts on the PFT populations and the PFT community. Indeed, both attributes are included as endpoints in the current OECD and USEPA guidelines, where effects on the survival of plants and the emergence of seedlings after exposure to a plant protection product are measured (Organisation for Economic Co-operation and Development 2006a, 2006b; US Environmental Protection Agency 2012a, 2012c). In contrast to the consequences of effects on seed sterility and plant mortality on population and community levels,

a reduction in seed production (lower number of produced seeds), establishment probability, or biomass had negative impacts on PFT populations and the community only under very high herbicidal effect intensities (i.e., high percentage reduction of the specific endpoint).

Several experimental studies have analyzed herbicide effects on reproductive attributes (Strandberg et al. 2012; Boutin et al. 2014; Schmitz et al. 2014). In contrast to our findings, Strandberg et al. (2012) concluded that seed production was a more sensitive (from the toxicological perspective) endpoint than biomass for some of the tested species. Nonetheless, the present study focuses on long-term population and community level effects, whereas Strandberg et al. (2012) investigated short-term effects at the individual level. Supporting our findings, Boutin et al. (2014) and Rotchés-Ribalta et al. (2015) found that the individual level sensitivity of reproductive endpoints relative to vegetative endpoints is species dependent.

There are only a few experimental (and no modeling) studies focusing on long-term effects (e.g., 3-yr study of Schmitz et al. 2014), which are considered particularly important to addressing the specific protection goals for nontarget terrestrial plants (European Food Safety Authority, Panel on Plant Protection Products and Their Residues 2010). Schmitz et al. (2014) could detect seed reduction and flower suppression for 3 out of 4 tested species after application of an herbicide at 30% of the field application rate. Even though the application rate far exceeds the expected off-field exposure rate under worst-case conditions, they were not able to observe an impact at the plant community level. It is necessary to show not only short-term, individual level effects but also to investigate long-term effects on the population and community. The comparison of the available experimental studies with our results clearly indicates that the IBC-grass model is a valuable tool for reducing the uncertainty associated with extrapolating conclusions from the short-term, individual level effects to the long-term, population level and community level effects.

Assuming the occurrence of generation carryover effects, in which case the F1 generation of a sprayed individual is still affected (e.g., emergence of seedlings is impacted in the P and F1 generations), the ecological sensitivity of each endpoint increased and significant impacts were detectable at lower effect intensities (see Figure 6). A sophisticated modeling approach based on the IBC-grass model, which includes realistic herbicide impacts as measured in the standard nontarget plant guideline studies (see Reeg et al. 2017) and in addition integrates potential generation carryover effects, could represent a good option to estimate potential ecological risks. Such an approach has the advantage of analyzing various potential scenarios—in this case various scenarios of generation carryover effects. In the event that the mode of action indicates potential generation carryover effects, an IBC-grass based higher tier evaluation might support the assessment of long-term risks to nontarget terrestrial plants. For the presented modeling exercise, it should be kept in mind that the inclusion of effects on the next generation (especially to the extent investigated for

the present study) is based on theoretical, worst-case assumptions that are not derived from actual experience and observation of such effects.

In summary, the current OECD and USEPA guidelines cover the ecologically most sensitive endpoints for protection of plant populations and communities—seed sterility and plant mortality. Seed production does not necessarily need to be investigated, as long as there is no indication that the herbicide has very strong effects at the individual plant level (>50%) in off-field areas. Considering the fact that seed dormancy and thus long-term seed banks (i.e., viable seeds within the soil) are not included in the current IBC-grass version, our model results are quite conservative. Long- as well as short-term seed banks can serve as buffer mechanisms for disturbances such as herbicide impacts. Reductions in seed production can be balanced by a seed bank from which seeds can establish, or locally extinct plant species are able to recolonize (Bakker et al. 1996). Thus, if at all, including a seed bank in our model would have led to lower effects than those shown.

## CONCLUSIONS

The extensive sensitivity analyses of potentially affected plant attributes at the individual level and the resulting long-term impact at the population and community level provide great insights into the current debate on the individual level sensitivity of reproductive versus vegetative endpoints and their consequences for plant communities in ecological risk assessment. The present study underlines the importance and the potential of modeling studies in risk assessments as a tool to indicate and predict ecologically relevant impacts of herbicides on nontarget terrestrial plant communities. At the same time, suitable empirical studies on a community level are needed to validate the model and thereby confirm and strengthen the model results. Validated community level modeling studies can support regulators in their decisions about the appropriate risk assessment endpoints and provide confidence in their assessments.

**Supplemental Data**—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4122.

**Acknowledgment**—The present study was funded by Bayer AG. We are grateful to K. Bergholz for his literature review on plant species occurring in field boundaries in Europe.

**Data Availability**—Data are available from the corresponding author (jreeg@uni-potsdam.de).

## REFERENCES

- Bagg J, Ball B, Banks S, Baute T, Böhner H, Brown C, Cowbrough M, Dyck J, Ferguson T, Follings J, Hall B, Hayes A, Johnson P, Kyle J, McDonald I, Moran M, Munroe J, Quesnel G, Rabe N, Rosser B, Spieser H, Stewart G, Tenuta A, Verhallen A. 2017. Weed control. In Brown C, Follings J, Moran M, Rosser B, eds, *Agronomy Guide for Field Crops*. Publication 811. Ontario Ministry of Agriculture, Food and Rural Affairs, Delhi, Ontario, Canada, pp 273–291.
- Bakker JP, Poschlod P, Strykstra RJ, Bekker RM, Thompson K. 1996. Seed banks and seed dispersal: Important topics in restoration ecology. *Acta Bot Neerl* 45:461–490.
- Barnthouse LW. 2004. Quantifying population recovery rates for ecological risk assessment. *Environ Toxicol Chem* 23:500–508.
- Bell T, Freckleton RP, Lewis OT. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecol Lett* 9:569–574.
- Berger U, Piou C, Schiffrers K, Grimm V. 2008. Competition among plants: Concepts, individual-based modelling approaches, and a proposal for a future research strategy. *Perspect Plant Ecol Evol Syst* 9:121–135.
- Boutin C, Lee HB, Peart ET, Batchelor PS, Maguire RJ. 2000. Effects of the sulfonylurea herbicide metsulfuron methyl on growth and reproduction of five wetland and terrestrial plant species. *Environ Toxicol Chem* 19:2532–2541.
- Boutin C, Strandberg B, Carpenter D, Mathiassen SK, Thomas PJ. 2014. Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? *Environ Pollut* 185:295–306.
- Carpenter D, Boutin C. 2010. Sublethal effects of the herbicide glufosinate ammonium on crops and wild plants: Short-term effects compared to vegetative recovery and plant reproduction. *Ecotoxicology* 19:1322–1336.
- Carpenter D, Boutin C, Allison JE. 2013. Effects of chlorimuron ethyl on terrestrial and wetland plants: Levels of, and time to recovery following sublethal exposure. *Environ Pollut* 172:275–282.
- DeAngelis DL, Mooij WM. 2005. Individual-based modeling of ecological and evolutionary processes. *Annu Rev Ecol Evol Syst* 36:147–168.
- Dáz S, Cabido M. 2001. Vive la différence: Plant functional diversity matters to ecosystem processes. *Trends Ecol Evol* 16:646–655.
- European and Mediterranean Plant Protection Organization. 2003. Environmental risk assessment scheme for plant protection products. *EPPO Bull* 33:195–209.
- European Food Safety Authority, Panel on Plant Protection Products and Their Residues. 2010. Scientific opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). *EFSA J* 8:1821–1875.
- European Food Safety Authority, Panel on Plant Protection Products and Their Residues. 2014. Scientific opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. *EFSA J* 12.
- Grimm V, Berger U, Bastiansen F, Eliassen S, Ginot V, Giske J, Goss-Custard J, Grand T, Heinz SK, Huse G, Huth A, Jepsen JU, Jørgensen C, Mooij WM, Müller B, Pe'er G, Piou C, Railsback SF, Robbins AM, Robbins MM, Rossmanith E, Rüger N, Strand E, Souissi S, Stillman RA, Vabø R, Visser U, DeAngelis DL. 2006. A standard protocol for describing individual-based and agent-based models. *Ecol Model* 198:115–126.
- Grimm V, Berger U, DeAngelis DL, Polhill JG, Giske J, Railsback SF. 2010. The ODD protocol: A review and first update. *Ecol Model* 221:2760–2768.
- Gross N, Bagousse-Pinguet YL, Liancourt P, Berdugo M, Gotelli NJ, Maestre FT. 2017. Functional trait diversity maximizes ecosystem multifunctionality. *Nat Ecol Evol* 1, 0132 DOI:10.1038/s41559-017-017-0.
- Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, van Groenendael JM, Klimes L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel AK, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E, Peco B. 2008. The LEDA traitbase: A database of life-history traits of Northwest European flora. *J Ecol* 96:1266–1274.
- Klimešová J, de Bello F. 2009. CLO-PLA: The database of clonal and bud bank traits of Central European flora. *J Veg Sci* 20:511–516.
- Klotz W, Kühn S, Durka I. 2002. BIOLFLOR—Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. In Klotz W, Kühn S, Durka I, eds, *Schriftenreihe Für Vegetationskunde* 38. Bundesamt für Naturschutz, Bonn, Deutschland, pp 1–334.
- Körner K, Pfestorf H, May F, Jeltsch F. 2014. Modelling the effect of belowground herbivory on grassland diversity. *Ecol Model* 273:79–85.
- May F, Grimm V, Jeltsch F. 2009. Reversed effects of grazing on plant diversity: The role of below-ground competition and size symmetry. *Oikos* 118:1830–1843.

- Organisation for Economic Co-operation and Development. 2006a. Test No. 208: Terrestrial plant test: Seedling emergence and seedling growth test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
- Organisation for Economic Co-operation and Development. 2006b. Test No. 227: Terrestrial plant test: Vegetative vigour test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
- Reeg J, Schad T, Preuss TG, Solga A, Körner K, Mihan C, Jeltsch F. 2017. Modelling direct and indirect effects of herbicides on non-target grassland communities. *Ecol Model* 348:44–55.
- Reynolds HL, Packer A, Bever JD, Clay K. 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84:2281–2291.
- Riemens MM, Dueck T, Kempenaar C. 2008. Predicting sublethal effects of herbicides on terrestrial non-crop plant species in the field from greenhouse data. *Environ Pollut* 155:141–149.
- Riemens MM, Dueck T, Kempenaar C, Lotz LAP, Kropff MJJ. 2009. Sublethal effects of herbicides on the biomass and seed production of terrestrial non-crop plant species, influenced by environment, development stage and assessment date. *Environ Pollut* 157:2306–2313.
- Rotchés-Ribalta R, Boutin C, Blanco-Moreno JM, Carpenter D, Sans FX. 2015. Herbicide impact on the growth and reproduction of characteristic and rare arable weeds of winter cereal fields. *Ecotoxicology* 24:991–1003.
- Schippers P, van Groenendael JM, Vleeshouwers LM, Hunt R. 2001. Herbaceous plant strategies in disturbed habitats. *Oikos* 95:198–210.
- Schmitz J, Hahn M, Brühl CA. 2014. Agrochemicals in field margins—An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agri Ecosyst Environ* 193:60–69.
- Shugart HH. 1989. *The role of ecological models in long-term ecological studies*. In Likens GE, ed, *Long-Term Studies in Ecology*. Springer Verlag, New York, NY, USA, pp 90–109.
- Stoyan D, Wagner S. 2001. Estimating the fruit dispersion of anemochorous forest trees. *Ecol Model* 145:35–47.
- Strandberg B, Mathiassen SK, Bruus M, Kjaer C, Damgaard C, Andersen HV, Bossi R, Løfstrøm P, Larsen SE, Bak J, Kudsk P. 2012. Pesticide Research No. 137: Effects of herbicides on non-target plants: How do effects in standard plant test relate to effects in natural habitats? Danish Ministry of the Environment. Copenhagen, Denmark.
- US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. 2012a. Seedling emergence and seedling growth. OCSPP 850.4100. *Ecological Effects Test Guidelines*. Washington, DC.
- US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. 2012b. Early seedling growth toxicity test. OCSPP 850.4230. *Ecological Effects Test Guidelines*. Washington, DC.
- US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. 2012c. Vegetative vigor. OCSPP 850.4150. *Ecological Effects Test Guidelines*. Washington, DC.
- Weiss L, Pfestorf H, May F, Körner K, Boch S, Fischer M, Müller J, Prati D, Socher SA, Jeltsch F. 2014. Grazing response patterns indicate isolation of semi-natural European grasslands. *Oikos* 123:599–612.