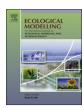
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Modelling direct and indirect effects of herbicides on non-target grassland communities



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ABSTRACT

Natural grassland communities are threatened by a variety of factors, such as climate change and increasing land use by mankind. The use of plant protection products (synthetic or organic) is mandatory in agricultural food production. To avoid adverse effects on natural grasslands within agricultural areas, synthetic plant protection products are strictly regulated in Europe. However, effects of herbicides on non-target terrestrial plants are primarily studied on the level of individual plants neglecting interactions between species.

In our study, we aim to extrapolate individual-level effects to the population and community level by adapting an existing spatio-temporal, individual-based plant community model (IBC-grass). We analyse the effects of herbicide exposure for three different grassland communities: 1) representative field boundary community, 2) *Calthion* grassland community, and 3) *Arrhenatheretalia* grassland community. Our simulations show that herbicide depositions can have effects on non-target plant communities resulting from direct and indirect effects on population level. The effect extent depends not only on the distance to the field, but also on the specific plant community, its disturbance regime (cutting frequency, trampling and grazing intensity) and resource level.

Mechanistic modelling approaches such as IBC-grass present a promising novel approach in transferring and extrapolating standardized pot experiments to community level and thereby bridging the gap between ecotoxicological testing (e.g. in the greenhouse) and protection goals referring to real world conditions.

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1. Introduction

Worldwide, the use of herbicides on conventionally managed arable fields is common practice for controlling weeds and safeguarding yields (Ecobichon, 2001; van der Werf, 1996; Wilson and Tisdell, 2001). Depending on wind conditions and application methods it is almost inevitable that small amounts of these herbicides spread into habitats in the vicinity of agricultural fields (field boundaries), i.e. non-target areas (de Snoo and van der Poll, 1999). Spray-drift is largely driven by spatial and temporal variability of environmental, ecological and agricultural conditions, e.g. the composition and structure of the landscape, weather events, spray-drift variability and application technology. Since herbicides are developed to control specific plant species considered as harmful within

an agricultural field, i.e. weeds, and have lethal effects on those target species, plant individuals occurring in field boundaries have a potentially high risk to be affected in a similar way when exposed to deposits (de Snoo and van der Poll, 1999; Kleijn and Snoeijing, 1997; Kleijn and Verbeek, 2000; Marrs et al., 1993). To mitigate exposure of those communities, measures like the use of drift reducing spray nozzles or consideration of buffer zones are regularly applied. However, such measures cannot always fully eliminate drift exposure.

Field boundaries are quite diverse. They include herbaceous field margins like ditches or river banks as well as hedges or forest edges. In some cases, meadows and grasslands are located in immediate proximity. Due to the use of fertilizers and management activities, field boundaries are characterized by a medium to high nutrient availability and disturbances such as trampling and mowing. Grassland communities are crucial for maintaining biodiversity within European landscapes. Natural grassland communities are threatened by climate change and increasing land use. Food and energy production by agriculture reduces the area available for

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semi-natural grassland communities. The use of plant protection products (synthetic or organic) is mandatory in agricultural food production. To avoid adverse effects on natural grassland communities within the agricultural areas strict regulations for synthetic plant protection products are in place in Europe and environmental risk assessments are conducted. The basis of these risk assessments are standardized biotests conducted at individual-level in the laboratory (e.g. OECD guideline studies (OECD, 2006a,b)). In contrast to the level of individual plants, the European Food and Safety Authority (EFSA) developed specific protection goals towards the protection on population and community level (EFSA, 2014). Specific protection goals for non-target terrestrial plants are primary production, nutrient cycling, water regulation, provision of habitat and food, among others. These goals can be met by protecting populations, functional groups, and/or communities considering diversity, population abundances, and/or biomass. Therefore, current individual-level OECD guidelines seem not to be sufficient to address these specific protection goals.

The scientific community has largely neglected to study species interactions, historically measuring the effects of herbicides on individual plants rather than communities (Dalton and Boutin, 2010). The number of existing experimental studies on the level of plant communities (de Snoo and van der Poll, 1999; Kleijn and Snoeijing, 1997; Marrs et al., 1993; Schmitz et al., 2014) is small which can mainly be attributed to the complexity of those trials with regard to variability, labour, and costs. Although duration of these community experiments available from the literature was up to three years, from the perspective of vegetation analysis the study periods were rather short. While longer experiments may provide new insights in plant community dynamics impacted by chemical stressors, the complexity of community-level experiments as well as the needed time and resources make it unlikely that empirical long term studies will be available in the future. Therefore, mechanistic computer models can provide an alternative approach to better understand non-target community effects. These mechanistic modelling approaches should be designed in a way that the available knowledge can be integrated and, hence, community interactions emerging from the models can be validated and tested. As stated above, most studies investigate the effects of herbicides on single plant species. In addition, competition of plant species depends on the direct neighbourhood. Therefore, the model approach should be individual-based and spatially explicit.

In our study, we adapt an existing spatio-temporal, individualbased plant community model (IBC-grass, Körner et al., 2014; May et al., 2009; Weiss et al., 2014) to analyse population and community level effects of herbicide exposure for grassland communities. IBC-grass simulates herbaceous plant community patterns on a local scale (patch of approx. 3 m²) by taking below- and aboveground interactions between individuals into account. To explore realistic herbicide effects at the individual-level we add a toxicological submodel to IBC-grass. The effect rate on the simulated vegetation patch is gained from a species effect distribution calculated by the exposure model Xplicit (Schad and Schulz, 2011; Schad 2013). Xplicit simulates herbicide exposure in field boundaries and calculates effect rates on plant individual-level depending on the specific spatial location of the patch in the landscape as well as on the ecotoxicological standard tests used for environmental risk assessment of herbicides in Europe (OECD, 2006a,b). We simulate the effect of herbicide exposures to three different plant communities: 1) a representative field boundary community, 2) a Calthion grassland community and 3) an Arrhenatheretalia grassland community. The communities differ in their regional species pool, management regime and resource level, which are determining factors for the composition and dynamics of grassland communities. We expect that (i) herbicide exposure will influence the interactions and resource competition between plant individuals and therefore cause direct and indirect effects on population and community level, and (ii) community response to herbicide exposure will depend on the specific species pool, management regime and resource level. For each community, we simulate local community patches at different distances to the treated arable field to account for effects of buffer zones as a potential mitigation measure.

2. Methods

2.1. Plant communities

A representative 'field boundary community' is based on a recent literature review on species found in such habitats in Europe (Kolja Bergholz unpublished, see in Appendix A, Table A.1 in Supplementary data for species list). In addition, we include two grassland communities with different management regimes and nutrient levels, which are common in Central Europe: (i) Calthion, which is a plant community that occurs in wet meadows with medium nutrient availability and (ii) a representative Arrhenatheretalia community that occurs in fertile meadows often used as pastures with high nutrient availability. To assess the species pool of those communities we reviewed vegetation surveys by Dierschke and colleagues (Dierschke, 2004; Dierschke et al., 2004; Fischer, 1985) and included expert knowledge (Michael Ristow, personal communication, see in Appendix A, Tables A.2 and A.3 in Supplementary data for species lists). The environmental conditions for these two communities differ in their nutrient availability and the amount of cutting events per year (Table 1).

2.2. The IBC-grass model

Plant community dynamics are driven by abiotic as well as biotic factors. Environmental characteristics such as nutrient levels, light and disturbances by either agricultural managements (e.g. by tractor crossings) or herbivory (e.g. grazing and trampling) determine the abiotic conditions in the community. Over time, plant individuals compete for resources and space. Thereby, the specific trait characteristics of a plant individual determine the growth potential and competitive strength.

IBC-grass is a well-established plant community model, which simulates local dynamics taking into account those main drivers. We base our approach on the published IBC-grass model of Weiss et al. (2014) enhancing it by adding an herbicide effect module and improving existing submodels. The flowchart (Fig. 1) gives a general overview of the considered processes in IBC-grass, main state variables can be found in Table 2. A detailed description of the base model and our modifications following the ODD protocol (Grimm et al., 2010, 2006) including all state variables and equations can be found in Appendix B in Supplementary data. In the following we give a summary of the main aspects, approaches and modifications in IBC-grass.

2.2.1. Trait-based approach

IBC-grass makes use of a trait-based approach, which classifies species into plant functional types (PFTs) according to their specific trait characteristics (Table 3), i.e. one PFT represents one or several plant species, which can be expected to respond in a similar way to abiotic and biotic conditions, e.g. resource competition, grazing intensities. Thus, general processes in grassland communities are captured whilst being transferable and general enough to allow conclusions for several grassland communities.

Overall, six different trait syndromes are considered in the standard IBC-grass model (Table 3). All plant species of the regional species pool (see Appendix A in Supplementary data) are parameterized using the trait data bases BiolFlor, LEDA and cloPla3 (Kleyer

Table 1
Summary of abiotic parameters of simulated plant communities. Field boundary represents a community based on a literature review by Kolja Bergholz; Arrhenatheretalia represents a grassland community with high disturbances by trampling and Calthion a meadow with few disturbance events to account for different disturbance intensities.

Belowground resources Disturbances	Field boundary high	Arrhenatheretalia high	Calthion medium	
Cutting per year [at 500 mg dw/cm ²]	1	3	1	
Trampling [% area/year]	10%	10%	2%	

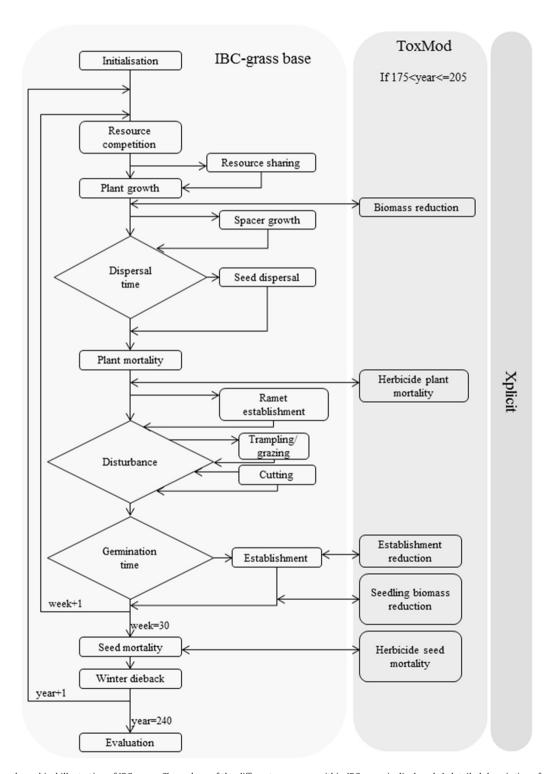


Fig. 1. Flowchart and graphical illustration of IBC-grass. Chronology of the different processes within IBC-grass is displayed. A detailed description of each process can be found in Appendix B in Supplementary data.

Table 2Main state variables of IBC-grass including a short explanation and the unit. More details can be found in the methods and ODD protocol.

Variables	Explanation	Unit	
type	PFT ID		
X _{coord} , y _{coord}	location of the plant's stem on the grid		
age	age of the plant	years	
m _{shoot/root/repro}	shoot/root/reproductive mass	mg	
W _{stress}	consecutive weeks of stress (i.e. lack of resources)	weeks	
SpacerLength	current spacer length	cm	
Spacerdirection	spacer direction		
Res _{A/B}	above- and belowground resources per cm ²	resource units	

Table 3Trait syndromes, PFT specific trait parameter values and used databases.

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

et al., 2008; Klimešová and de Bello, 2009; Klotz et al., 2002, see also Table 3). Thereby, each plant species can be classified into a PFT. An overview of the plant species and their classification to PFTs can be found in Tables A.1–A.3 in Supplementary data.

2.2.2. Spatial dimensions

IBC-grass simulates plant community dynamics on a local patch of approx. 3 m^2 . The local patch is divided by 173×173 grid cells of 1 cm² each. Periodic boundary conditions are used to avoid edge effects. Two layers are distinguished in IBC-grass: aboveground and belowground.

Each grid cell can fit the stem of one plant. The position of a plant's stem is defined by the state variables x_{coord} and y_{coord} . In an aboveground and in a belowground layer the plants' roots and shoots cover a circular area around its stem ($A_{shoot/root}$, Table 4) ('Zone-of-Influence' (ZOI)). The above and belowground ZOIs are determined by the specific root and shoot mass ($m_{shoot/root}$), root and shoot geometry ($c_{shoot/root}$), and the growth form of the plant individual ($f_{leaf/root}$). ZOIs of neighbouring plant individuals can overlap. Within these overlapping zones, plants compete for light (aboveground) and belowground resources (see Section *Interactions and growth*).

In contrast to established plants, several seeds can be located in one grid cell. Like with plants, the location of a seed is stored by their x and y coordinates (x_{coord} , y_{coord}). Seeds can only germinate and establish if the cell is not covered by the aboveground ZOI of any other plant individual. Seed dispersal distance is dependent on

seed mass, and drawn from a log-normal and the direction from a uniform distribution (Stoyan and Wagner, 2001).

2.2.3. Interactions and growth

Each plant is defined by its current size, neighbourhood, and specific trait characteristics, such as competitive strength and growth potential. Plant individuals compete for resources within the overlapping regions of their ZOI (Table 4). Aboveground, asymmetric competition is simulated such that taller individuals (as resulting from their current aboveground biomass and their growth form) will acquire more light than smaller individuals (Table 4).

Plant growth depends on the constant conversion rate g of resources into biomass, the acquired resources Δres , the current shoot mass and the specific trait characteristics of the shoot c_{shoot} , growth form f_{leaf} , maximal resource utilization ru_{max} , and the maximal plant mass m_{max} . In the absence of competition, plants show a sigmoid growth (Table 4, Eq. 'shoot/root growth', see also in Berger et al., 2008; DeAngelis and Mooij, 2005). Lateral plant growth is considered for PFTs exhibiting clonal growth. One spacer can grow per clonal PFT individual. 5% of the acquired resources are allocated to the growth of the spacer. The direction in which the spacer grows is chosen randomly from a uniform distribution. The distance of spacer growth is randomly chosen from a normal distribution based on the type-specific mean distance (SpacerL). If the determined distance the spacer has to grow is reached and the respective cell is not the centre of another individual, spacer growth stops and the new ramet can establish with a fixed probability (p_{ram}). If the reached

Table 4Main equations in IBC-grass regarding competition, growth and mortality. More details can be found in the methods and ODD protocol.

Process	Equation	Variables
zone of influence (ZOI)	$A_{shoot} = c_{shoot} \cdot (f_{leaf} \cdot m_{shoot})^{2/3}$	c_{shoot} — PFT specific leaf area
	$A_{\text{root}} = RAR \cdot (\cdot m_{\text{root}})^{2/3}$	RAR – root form (1)
resource competition	$\Delta res_i = \frac{\beta_i}{r} \cdot Res_{cell}$	f_{leaf} – PFT specific growth form
	<u>"</u>	$m_{shoot/root}$ – shoot/root mass [mg dry weight]
	$\sum_{j} \beta_{j}$	Res _{cell} – resources in cell
	j=1	ru_{max} – PFT specific maximal resource utilisation
asymmetric competition	8 - ru m · f1	g – conversion rate resources to biomass
symmetric competition	$\beta_i = ru_{max} \cdot \frac{1}{m}$	n_{PFT} – number of neighbouring PFTs
1	/n _{pFT}	m_{max} – PFT specific maximal plant mass [mg dry weight]
shoot growth	$\Delta m = g \cdot \left(\Delta res - c_{shoot} \cdot f_{leaf}^{2/3} \cdot ru_{max} \cdot \frac{m_{shoot}^2}{4/3} \right)$	w _{stress} consecutive weeks under stress
	m _{max} ^{4/3}	surv _{max} – PFT specific maximal survival under stress [weeks]
root growth	$\Delta m = g \cdot \left(\Delta res - RAR \cdot ru_{max} \cdot \frac{m_{root}^2}{m_{root}^2} \right)$	p_{base} – base mortality (0.7%)
density dependent	$p = p + \frac{W_{\text{stress}}}{W_{\text{stress}}}$ with	current_abundance – current abundance of a PFT
mortality	$\begin{split} & \beta_{i} = ru_{max} \cdot \frac{1}{\sqrt{n_{PFT}}} \\ & \Delta m = g \cdot \left(\Delta res - c_{shoot} \cdot f_{leaf}^{2/3} \cdot ru_{max} \cdot \frac{m_{shoot}^{2}}{m_{max}^{4/3}} \right) \\ & \Delta m = g \cdot \left(\Delta res - RAR \cdot ru_{max} \cdot \frac{m_{root}^{2}}{m_{max}^{4/3}} \right) \\ & p_{mort} = p_{base_new} + \frac{W_{stress}}{surv_{max}} & with \end{split}$	max_abundance - maximal potential abundance of a PFT (assuming
inortanty		fully grown individuals)
	$p_{base_new} = p_{base} \cdot (1 + e^{\frac{5*current_abundance}{max abundance}})$	

cell is occupied by the centre of a different plant, spacer growth continues randomly within a radius of two cells.

2.2.4. Mortality

Plant individuals die due to consecutive weeks of resource stress (trait dependent), stochastic background mortality (density dependent), or during winter dieback. 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. Additionally, we added density dependent background mortality to the base background mortality of 0.7% per week corresponding to an annual mortality rate of 20 % (Schippers et al., 2001). To avoid unrealistic population densities background mortality increases exponentially if the abundance of a PFT reaches the maximum possible abundance of fully-grown individuals (i.e. a monoculture), (s. Appendix B in Supplementary data, ODD protocol). During winter, the aboveground biomass of each plant is decreased by 50% simulating winter dieback. If the biomass of a plant is below 10 mg, the plant is considered dead and is removed from the grid. Seeds have a probability of 50% to die during winter.

2.2.5. Abiotic factors

Above- and belowground resources (e.g. nutrients and light) are distributed homogenously in space and time, i.e. we model no gradients of abiotic factors within the simulated 3 m² patch nor temporal changes of abiotic factors within the growing period. IBC-grass includes biotic disturbances such as grazing, trampling or cutting (see ODD-protocol for detailed information).

2.2.6. Temporal dimensions

One time step in IBC-grass represents one week with a total of 30 growth weeks (i.e. vegetation period) simulating one year. At the beginning of a simulation, it takes several years until a stable PFT community is reached. This is mainly explained by environmental filtering and competition between PFTs. After 150 years, the PFT community is within a stable state. As a reference, we run the model for another 25 years before we start simulating herbicide exposure effects in year 175.

2.3. Modelling herbicide exposure in the landscape – Xplicit

Schad and Schulz (Schad and Schulz, 2011; Schad, 2013) developed a model, Xplicit, which simulates herbicide exposure over time and space taking into account different use rates, wind directions, application technologies and landscape structures. In our approach, we use Xplicit to simulate the drift of a broad spectrum herbicide (i.e. non selective, affecting a broad range of weed species), which has a half-life period (DT50) of 10 days, i.e. after

10 days half of the herbicide is decomposed. The typical use pattern consists of two applications per year: one application with a field dose of 739.76 g/ha and a second application with a field dose of 594.05 g/ha. Xplicit derives the variability of spray-drift deposition (distance dependent) from drift models. In our approach, we use the established drift model AgDRIFT (Bird et al., 2002; Hewitt, 2002; Hewitt et al., 2002; Teske et al., 2002), which is frequently used in risk assessments for instance by US Environmental Protection Agency. AgDRIFT is a complex drift model containing several options for calculating drift by e.g. aerial, ground and orchard application. It considers e.g. droplet size distribution and boom height (Hewitt, 2002). As a worst-case scenario, west-wind conditions are assumed, i.e. wind is going directly into the neighbouring field boundary, without drift reducing technologies. No special landscape structures, e.g. slopes are considered. The simulated herbicide exposure in the landscape is variable in space but constant over the years. All plants in the field boundary community receive full spray-drift deposition (i.e., no 'in-community' variability due to spray-drift filtering e.g. higher leaf layers intercepting herbicide before it reaches lower leaf-layers). The herbicide distribution within one IBC-grass patch is homogenously.

2.3.1. Calculating individual-level effects based on herbicide exposure

In standard ecotoxicological dose-response tests (OECD, 2006a,b) the effect of certain herbicide loads on seed mortality and seedling growth (survival, dry weight and emergence) and vegetative vigour (survival, dry weight, shoot height) are tested for a defined number of selected plant species (OECD, 2006a,b). Based on this data, the effects on a given tested species under various doses are estimated by dose-response curves (OECD, 2006a,b). Combining the maximum herbicide exposure within a year on a patch in the landscape (as calculated by Xplicit) and the dose-response curves, we are able to calculate species effect distributions (SEffDs): The tested species are ranked and plotted over their effects for a given herbicide dose. In doing so, we are able to calculate individuallevel effects on off-field patches within the agricultural landscape context (Fig. 2 as example, SEffDs of all simulated patches and endpoints can be found in Appendix C, Fig. C.1 in Supplementary data). Our tested broad spectrum herbicide shows strongest effects on biomass, followed by effects on survival. Emergence shows lowest effect rates. We selected 6 patches within a simulated landscape differing in their distance to the field boundary (1–6 m). The specific maximum herbicide loads in the different distances are presented in Table 5.

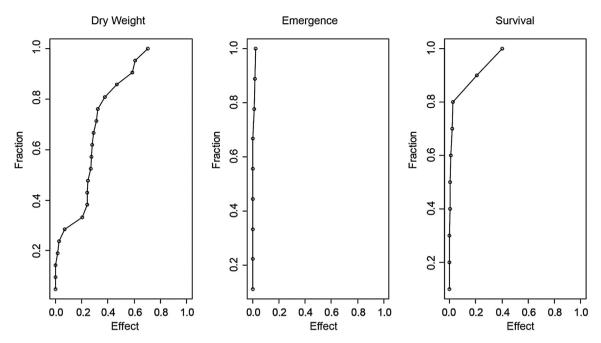


Fig. 2. Example of species effect distributions (SEffDs) for attributes 'Shoot Dry Weight', 'Emergence' and 'Survival'. SEffDs are based on the standardized greenhouse experiments, in which at least 6 plant species (selected of a list of suitable monocotyledonous and dicotyledonous plant species (OECD, 2006a,b)) are oversprayed with different herbicide rates. Taking into account the simulated herbicide exposure on a patch in the off-field landscape, herbicide effect rates ('Effect', x-axis) can be calculated. The SEffD graphs show the cumulative effect distributions of selected species ('fraction', ordinate): e.g. for 80% of the species reduction in dry weight is ≤40%. Herbicide susceptibility is synonymous to this fraction. A PFT with a susceptibility of 0.8 will get an effect rate on dry weight of 0.4. Graphs show SEffDs for a patch in direct neighbourhood to the agricultural field.

Table 5Maximum herbicide load each year within the different patches according to Xplicit calculations.

Maximal herbicide load/year [g/ha]
72.149
32.784
20.266
14.324
10.916
8.732

2.3.2. Herbicide exposure effects in IBC-grass

To include the effects of herbicide exposure on plant individuals in IBC-grass, we extend the model with toxicological sub-processes. In this way, growth, mortality and establishment of seedlings and adult plants are influenced by the herbicidal effect rates in accordance with the SEffDs based on OECD guideline endpoints (OECD, 2006a,b) taking into account PFT specific herbicide susceptibilities (see 2.3.2.1).

- After normal plant growth is calculated, biomass is reduced by the specific effect rate.
- After plants suffered from the stress-induced and/or demographic-related mortality, additional herbicide-induced mortality is included with the specific probabilistic effect rate
- Seedling mortality is increased by the specific probabilistic effect rate
- Seedling biomass is reduced by the specific effect rate after the seedling is established.
- After seeds suffered from the demographic-induced mortality, additional herbicide-induced mortality occurs with the specific probabilistic effect rate

In the standard non-target terrestrial plant studies for the herbicide used in this modelling exercise, effects on dry weight and shoot length are measured (acc. to OECD and OCSPP Guidelines

(EPA, 2012a,b; OECD, 2006a,b)). However, in IBC-grass we do not consider shoot length directly, but indirectly by correlating biomass and growth form in the ZOI approach. In order not to underestimate the effect, we select the most sensitive endpoint which is dry weight in our case study. Furthermore, due to the way growth is calculated in the model, it was not possible to relate the effect on dry weight to a growth rate (which would directly reflect the outputs of the guideline studies) but the effect has to be concentrated into one week and affected biomass directly. Therefore, effects on biomass can be assumed as very conservative and potentially overestimated.

2.3.2.1. Species specific susceptibilities. Herbicide susceptibility varies among different plant species, e.g. due to secondary metabolites, specific plant features like hairiness of leafs (Prather et al., 2000) or the mode of action of the compound. A broad range of plant species would need to be tested for each compound. This is partially fulfilled by conducting standard non-target terrestrial plant guideline studies but to further account for the wide variety of plants those tests would become very time-consuming, expensive and labour intensive. In addition, the handling of some plants is not practical or the tests are not feasible (e.g. unsuitable growth form such as ranking plants, high variation, low germination probability, etc). Therefore, only selected plant species are considered for standardized greenhouse experiments, which are deemed appropriate to cover the range of possible sensitivities when an assessment factor is considered (Christl, 2015).

This also holds true for our case study compound, where doseresponse tests were conducted for standard test species only. Therefore, we are not able to assign any verified herbicide susceptibilities to our PFTs. As a temporary conservative work-around we assign herbicide susceptibility randomly among the PFTs in our regional pool. We use a uniform distribution of herbicide susceptibility varying between 0 and 1. For each Monte Carlo run, herbicide susceptibility is assigned anew to cover a maximum range of possible susceptibility distributions. The resulting high variability allows for the identification of general response pattern that do not depend on specific susceptibility distributions.

2.4. Simulation scenarios and analyses

We simulate worst-case scenarios, in which herbicide exposure occurs over a period of 30 years (1 application per year) under conservative conditions, i.e., during the application of the herbicide on the arable field wind is coming directly towards the examined field boundary, and no drift-reducing technologies are applied. To account for potential recovery effects, these 30 years are followed by a period of 35 years without any herbicide exposure. A scenario without any herbicide exposure serves as control. Each scenario is repeated 50 times (i.e. 50 Monte Carlo runs (MCs)) to account for the high stochasticity of the model.

We analyse the effects on (i) population size on population level within community context and the effects on (ii) aboveground biomass, (iii) PFT richness and (iv) diversity on community level. The following analyses are conducted:

For each patch and evaluation variable (see above), the mean (per week and year) of the control scenario is calculated and used as a reference for standardization. The values of all evaluation variables of all 50 treatment and 50 control MCs are standardized by the reference (per week and year). For each year, the 2.5th and 97.5th percentile of the standardized control MCs are selected as a measure of standard variation of population size, aboveground biomass, PFT richness and diversity within the community. On community level, standardized values of treatment MCs are plotted per year in boxplots. If the median is out of the range of standard variation, we consider it a significant effect (which should not to be confused with the concept of statistical significance). On population level, we plot the mean of all standardized treatment MCs per week and year to show the temporal dynamics within and between years.

3. Results

3.1. Effects on community level and differences between plant communities

Looking at the behaviour of community-level evaluation variables in the course of time for one scenario of the simulated Calthion community, aboveground biomass is significantly decreased during the week of herbicide drift exposure and recovers over the year i.e. one growing period (Fig. 3). However, it is not completely recovering each year resulting in a slight decrease of aboveground biomass between the years. Only minor effects can be observed for PFT richness: it slightly increases after herbicide exposure but decreases over the years. However, the variation within the control simulations indicates that these effects are within the normal variation of PFT richness in the presented community. In contrast, strong effects can be detected on diversity (represented via the inverse Simpson index, taking into account PFT richness and abundance). There is a strong decline within the presented time frame. However, the index shows a relatively high fluctuation already in years without herbicide exposure.

Communities differ in their natural variability; the field boundary community and *Arrhenatheretalia* community display more variability than the *Calthion* community indicated by more outliers in periods without simulated herbicide treatment (year > 30) (Fig. 4). Strongest effects can be observed in the *Calthion* community for PFT richness and inverse Simpson index. Diversity is significantly decreased (i.e. boxplots outside of ribbon). Field boundary community and *Arrhenatheretalia* community show similar effects of simulated herbicide drift exposure. Aboveground biomass is strongly decreased in few weeks per year (represented by a higher

amount of outliers (one outlier = one week within a treatment simulation), but is not significantly decreased in the long-term. Aboveground biomass recovers completely if herbicide treatment is suspended. In addition, PFT richness is slightly decreased by herbicide drift exposure. Effects are not significant for all simulations and the whole year, i.e. PFT richness is able to almost recover within a year. But the trend of the 30 years of repeated herbicide exposure suggests that there probably will be significant long-term effects on the PFT richness (median values constantly decreasing). Nevertheless, PFT richness is able to recover within a few years of suspended herbicide exposure. However, significant effects are detected for diversity, which not only considers PFT richness, but also abundance of PFTs. For the field boundary community, inverse Simpson-index is first strongly declining, but able to steadily recover. Considering the decline in PFT richness, this indicates a shift in community composition. Within the Arrhenatheretalia community, significant effects can be detected on biodiversity, but there are high fluctuations even when herbicide treatment is suspended. Therefore, the field boundary community has a slightly higher recovery potential than the Arrhenatheretalia community.

3.2. Effects on population level

Since *Calthion* shows highest sensitivities, population level results are shown only for *Calthion*; however, results for other communities can be found in the Appendix C, Figs. C.2 and C.3 in Supplementary data.

As expected, all PFTs show an immediate negative response to herbicide exposure (e.g. peaks at the beginning of each year, Fig. 5). Over the long term, three PFTs show a decline in population sizes over the year (PFT Agrostis canina, Bistorta officinalis, Cirsium rivulare and Carex panacea). However, in four cases (PFT Galium uliginosum, Ranunculus bulbosus, Senecio aquaticus and Taraxacum officinale agg.), the mean standardized population size increases over time, i.e. the PFT recovers from the immediate herbicide effect within the year and even more individuals are able to establish in the community. In this respect, also infrequent PFTs show several occurrences where standardized population sizes strongly increase during some weeks (see Appendix C, Fig. C.4 in Supplementary data).

3.3. Distance to field

The effect of herbicide drift exposure strongly decreases within the first meter distance to the arable field (1–2 m). In the adjoining 2–6 m distance, there is only a slight reduction of effects (Fig. 6). Here, herbicide exposure is already diminished.

4. Discussion

Our simulation scenarios demonstrate that herbicide depositions can have complex effects on non-target plant communities. These effects differ for the different communities. We test three plant communities in nutrient rich to medium environments with high to medium disturbance levels by mowing and trampling activities. Among the simulated communities, the *Calthion* community shows the highest sensitivity towards herbicide deposition. A *Calthion* grassland is characterized by medium to low nutrient levels and is typically managed as a meadow with only one cut per year (Dierschke et al., 2004). Therefore, plant species occurring in such grasslands are rather poorly adapted to disturbances. The main driver in such systems is the adaptation of plants to comparably low resource levels (Aerts, 1999). Introducing herbicide deposition to these communities causes loss of biomass and increases mortality, similar to effects of increased grazing and mowing. In response,

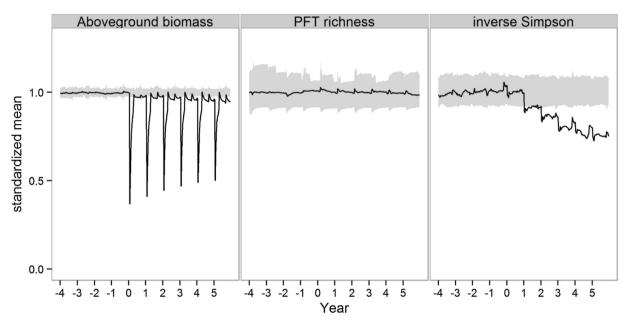


Fig. 3. Timeline of herbicide drift exposure effects on community-level for Calthion community on a patch in 1 m distance. The continuous line represents the mean of standardized values (by the weekly mean of 50 control simulations) of each week per year for 50 treatment simulations. A standardized value of 1 means, that there is no difference between control and treatment. Grey background ribbons showing the 2.5th and 97.5th percentile of standardized control simulations. Graphics show 4 years before treatment and the first 6 years of simulated herbicide exposure (starting at year 0). I.e. a decrease by 0.5 means an average reduction e.g. in aboveground biomass of 50.9%

plant species specialized to low disturbed but resource-stressed environments are replaced by species that are able to recover faster from disturbance events. Contrastingly, the field boundary plant community and *Arrhenatheretalia* grassland are characterized by high nutrient levels (due to fertilizer deposition/input) and higher disturbance events (e.g. farmer's activities on a field boundary and three cutting events on an *Arrhenatheretalia* meadow (Dierschke, 2004; Fischer, 1985)). The main aspect, which drives the dynamics of these plant communities are disturbance events, since resources are not the limiting factor (Aerts, 1999). Thus, the addition of herbicide deposition as another disturbance agent does not change species composition over the long-term. Frequent plant species in these communities are already adapted to high disturbances and therefore, can cope better with herbicide deposition events than species in a less disturbed community such as *Calthion*.

The indirect effects of herbicide deposition, which result in the change of species composition, can best be observed on population level. Over the long-term, single PFTs show either a negative, positive, or neutral effect on population size. During the herbicide exposure in the period of germination, all PFTs show a negative effect. However, as the year progresses, population sizes show different trends. For example, PFTs Agrostis canina, Bistorta officinalis, Carex panicea and Cirsium rivulare are not able to recover completely within a year. Consequently, their population sizes are decreasing over the years. All of these PFTs include plant species with medium to large maximal plant sizes, semi-rosette growth forms and adaptations to resource stress. These species can survive low resource uptakes for a longer time period. However, the combination of high maximal plant sizes and a semi-rosette growth form results in lower growth rates as a trade-off. Plant individuals of these PFTs need longer to rebuild biomass after a disturbance event and, in addition, produce fewer seeds (with higher seed masses) (Grime, 2001). Although seeds with higher seed masses have a competitive advantage in establishment (Schippers et al., 2001), they also have lower chances in dispersing into empty establishment sites as they have smaller dispersal kernels. Therefore, replacing deceased individuals with new seedlings is a low probability. In contrast, population sizes of the PFTs Galium uliginosum, Ranunculus bulbosus, Senecio aquaticus and Taraxacum officinale increase over the years. These PFTs are characterized by small plant sizes and show no adaptation to resource stress. Correlated to their small plant sizes, these species produce many small seeds, which have a wide dispersal range. Therefore, there is a higher chance for seedlings to establish, hence, compensating the increased mortality of single individuals.

Herbicide deposition effects on plant communities are not only depending on the mode of action of the compound, management, species pool and certain traits, but also on the distance to the arable field. The fraction of herbicide in a certain distance to the arable field depends on the herbicide-specific application method, the wind direction and intensity and the distance to the last nozzle (Wang and Rautmann, 2008). Therefore, on patches directly located at the field boundary, a higher herbicide deposition can be observed and, correspondingly, also stronger negative effects on the community. A distance of 3–6 m is often considered as a buffer zone in herbicide risk assessments (de Snoo, 1999). This is in accordance with the findings of our simulation experiment. At a distance of 3m, IBC-grass detects only negligible effects on community level (Fig. 6).

Spray drift interception by taller plants or other exposure routes, e.g. vapour drift or run-off, could be other important mechanisms of individual plant exposure that could have impacts on relative PFT population responses and thus also on community composition and response patterns. Implementing this additional variability into the IBC-grass would add an additional level of complexity. Since the current version of IBC-grass mainly aims at a first basic demonstration of its potential to scale up from individual effects to populations and communities these and other additional aspects will be investigated in a future version of the model.

Due to the lack of information on PFT specific herbicide susceptibilities, we assign this parameter randomly in each Monte Carlo run (MC), i.e. the complete range of PFT susceptibility is sampled. Therefore, variability between MCs is strongly increased and one could expect that effects seen in single runs level each other (e.g. MCs in which a certain PFT suffers less compensates for MCs in which this specific PFT suffers more due to assignment of higher herbicide susceptibility). However, in spite of these artificially assigned sus-

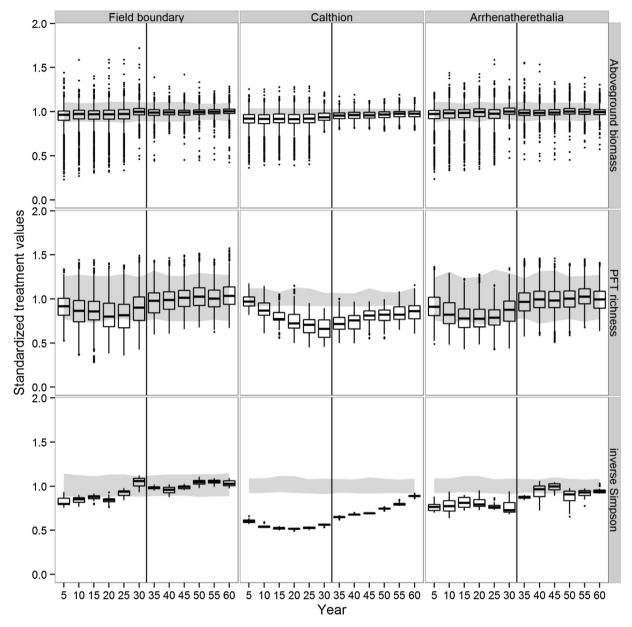


Fig. 4. Differences in communities regarding herbicide drift exposure effects on community-level for total aboveground biomass, PFT richness and inverse Simpson index on a patch in 1 m distance. Boxplots represent standardized values (by the weekly mean of 50 control simulations) of each week per plotted year for each of the 50 treatment simulations (i.e. one boxplot consists out of 1500 standardized values). For reasons of readability only every 5th year is plotted. A standardized value of 1 means, that there is no difference between control and treatment. All outliers are presented. Grey background ribbons showing the 2.5th and 97.5th percentile of standardized control simulations. Herbicide treatment was simulated for 30 years, as indicated by the vertical line, followed by 35 years of potential recovery (without herbicide treatment).

ceptibilities, several PFTs show a clear positive or negative feedback on herbicide deposition. This indicates that the IBC-grass model is rather robust to detect possible herbicide deposition effects although species specific herbicide susceptibilities remain uncertain. In other words the impact on species in a community is more related to their life-strategy and the resulting traits than to their specific sensitivity to the pesticide. However, the understanding of the plant species' susceptibility to herbicides should be deepened and enlarged. Future studies should focus on plant traits that may lead to lower or higher susceptibilities (see Rubach et al., 2010 for a conceptual approach). Boutin et al. (2012) studied the relationship between herbicide efficiency and certain plant traits, which potentially influence the efficiency. They were not able to find any correlation between the measured traits and the efficiency. However, they agreed that further research is needed since they only tested a few of several traits, which might influence herbicide sus-

ceptibility. In IBC-grass, future modelling studies should also focus on grouping PFT herbicide susceptibility according to specific trait characteristics, e.g. distinguishing between monocots and dicots.

In addition, we face a general problem in translating the effects on the biomass into IBC-grass. Within standard ecotoxicological studies, effects on dry weight are measured 7, 14 and 21 days after exposure at the 2–4 leaves growth stage. Effects on older plant individuals are not investigated, since they are assumed to be covered by the more sensitive juvenile stages. However, also plant individuals in advanced growth stages will experience effects on biomass. Due to a lack of data on older individuals within the ecotoxicological experiments, we apply the full effect extents on all plant individuals, disregarding the individual, plant age specific growth rate. Therefore, older plant individuals might be assigned a greater effect than in the real-world. Eventually, further research is needed to

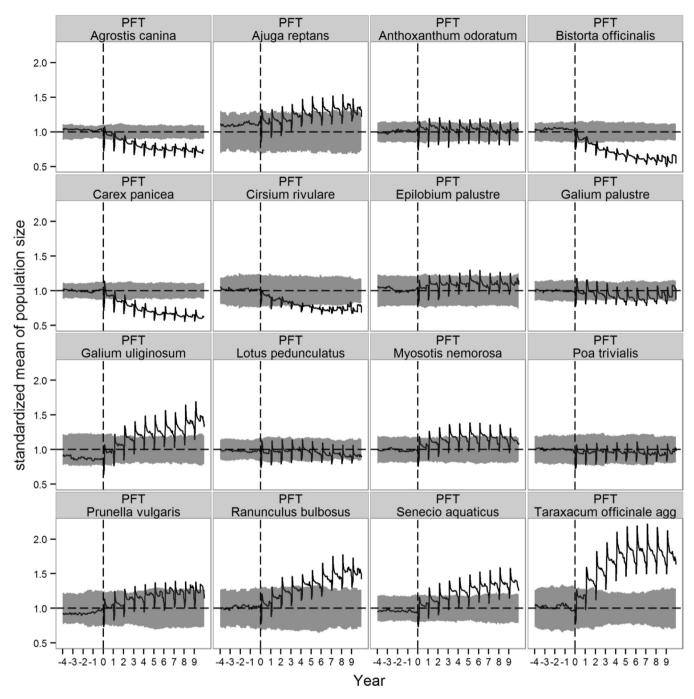


Fig. 5. Herbicide drift effects on PFT population sizes within community context for Calthion community on a patch in 1 m distance (other communities shown in Appendix C, Fig. C.2 and C.3 in Supplementary data). In this case the PFTs are represented by a certain plant species which belongs to the PFT group. Solid lines represent the standardized mean (standardized by the weekly mean of 50 control simulations) of each week per year out of 50 treatment simulations. A standardized mean of 1 means, that there is no difference between control and treatment. Grey background ribbons showing the standard deviation of standardized control simulations. For a better visualization of the temporal dynamics, only the last 5 years before herbicide drift exposure and 10 years during herbicide exposure and only PFTs with a mean frequency (in control simulations) of >0.9 are presented. Population dynamics over the whole simulated period (30 years herbicide exposure followed by 35 years 'recovery' without exposure can be found in the Appendix Fig. C.5 in Supplementary data).

investigate the decrease in effect extent for older plant individuals for more realistic effect patterns.

Several studies conducted experiments to test the effects of various herbicides on plants with different modes of actions (e.g. Damgaard et al., 2011; Pfleeger et al., 2012; Riemens et al., 2008). These studies focused either on the differences between crops and weeds (e.g. Carpenter and Boutin, 2010), sublethal and reproductive effects (e.g. Wagner and Nelson, 2014) or the sufficiency of standardized regulatory risk assessment tests (e.g. Kleijn and

Snoeijing, 1997). However, few studies tested effects on community level in realistic field/(semi-)field experiments. Kleijn and Snoeijing (1997) did a field experiment over two years. Similar to our simulation experiments, they detected different herbicide exposure effects for different plant communities especially due to species composition and nutrient levels. However, the authors highlighted that management regimes (mowing, grazing) may be of more importance than herbicide exposure. Since we regard different plant communities with different management regimes, we

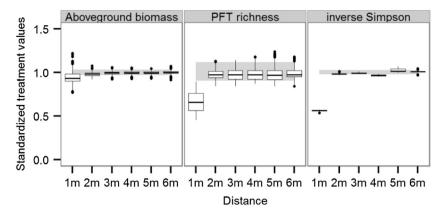


Fig. 6. Drift exposure effects on community level of the Calthion community at varying distances to the arable field after 30 years of simulated herbicide application. Boxplots represent standardized values (by the weekly mean of 50 control simulations) of each week per year for each of the 50 treatment simulations (i.e. one boxplot consists out of 15,000 standardized values). The graphic shows the last year of 30 years repeated herbicide application. A standardized value of 1 means, that there is no difference between control and treatment. All outliers are presented. Grey background ribbons showing the 2.5th and 97.5th percentile of standardized control simulations. The x-axis 'distance' indicates the potential herbicide exposure extent according to Xplicit calculations. However, community dynamics in IBC-grass are still simulated on a 3 m² patch.

are able to confirm that management regimes might indeed be a decisive factor for the composition of plant communities in cultivated landscapes, dominating over the effect of herbicide exposure on a plant community. Marrs and Frost (1997) did a microcosm approach with standardized species assemblages which lasted for 3 years. They found similar patterns compared to our results. With increasing distance, herbicide drift effects were diminished and they found positive feedbacks of some species, which was related to reduced competitive pressure. More recent studies also found similar results (e.g. Damgaard et al., 2014; Schmitz et al., 2014). However, all of these studies were carried out for not longer than three years. Real long-term effects and trends could not be observed. Kleijn and Snoeijing (1997) even mentioned the high variability between years. Our results show that the intensity of effects is increasing over time and potential risk of extinction for some PFTs can be only seen after several years. Model approaches have the advantage to incorporate long-term investigations and higher amount of repetitions, thereby capturing the high natural variability of real-world plant communities and long-term effects of herbicide drift exposure.

5. Conclusion

In this study, we give a first impression of the potential and possibilities of using a spatially-explicit, plant-functional and individual-based modelling approach to extrapolate individuallevel effects of non-target herbicide exposure measured in greenhouses to plant community level within the landscape. Using simulated effects of a broad spectrum herbicide, which acts on all plant species, as a first general application we show how effects on plant individuals can lead to both direct and indirect community responses by modifying population dynamics and relative interaction networks. The chosen trait- and individual-based modelling approach will also allow including and exploring more refined scenarios (e.g. selective herbicides, which act only on monocots or dicots) and more detailed processes (e.g. increased herbicide interception by taller plants) in future versions of IBC-grass. In part, this will have to be accompanied by additional experiments such as susceptibility measurements for a broader range of herbaceous

Overall, mechanistic modelling approaches such as IBC-grass can help to improve our understanding of the complex interactions within grassland communities. We show that they also present a promising approach in transferring and extrapolating standardized pot experiments (as regulated in e.g. the OECD guidelines 208

and 227 (OECD, 2006a,b)) to population and community level and thereby bridging the gap between ecotoxicological testing (e.g. in the greenhouse) and protection goals referring to real world conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolmodel.2017. 01.010.

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