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# Quantifying the impact of above- and belowground higher trophic levels on plant and herbivore performance by modeling

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Growing empirical evidence suggests that aboveground and belowground multitrophic communities interact. However, investigations that comprehensively explore the impacts of above- and belowground third and higher trophic level organisms on plant and herbivore performance are thus far lacking. We tested the hypotheses that above- and belowground higher trophic level organisms as well as decomposers affect plant and herbivore performance and that these effects cross the soil–surface boundary. We used a well-validated simulation model that is individual-based for aboveground trophic levels such as shoot herbivores, parasitoids, and hyperparasitoids while considering belowground herbivores and their antagonists at the population level. We simulated greenhouse experiments by removing trophic levels and decomposers from the simulations in a factorial design. Decomposers and above- and belowground third trophic levels affected plant and herbivore mortality, root biomass, and to a lesser extent shoot biomass. We also tested the effect of gradual modifications of the interactions between different trophic level organisms with a sensitivity analysis. Shoot and root biomass were highly sensitive to the impact of the fourth trophic level. We found effects that cross the soil surface, such as aboveground herbivores and parasitoids affecting root biomass and belowground herbivores influencing aboveground herbivore mortality. We conclude that higher trophic level organisms and decomposers can strongly influence plant and herbivore performance. We propose that our modelling framework can be used in future applications to quantitatively explore the possible outcomes of complex above- and belowground multitrophic interactions under a range of environmental conditions and species compositions.

Aboveground and belowground multitrophic interactions have long been studied in isolation with an emphasis on aboveground interactions. However, it is now increasingly acknowledged that above- and belowground multitrophic communities interact through shared host plants (Masters et al. 1993, Gange and West 1994, Scheu 2001, Van der Putten et al. 2001, Bezemer et al. 2002, Poveda et al. 2003, 2005, van Dam et al. 2003, Wardle et al. 2004, Soler et al. 2005, Fukami et al. 2006). The majority of the published above-belowground multitrophic interaction studies are limited to bitrophic interactions exposing plants to herbivores, pathogens or symbiotic mutualists (Moran and Whitham 1990, Masters et al. 1993, Müller-Schärer and Brown 1995, Rudgers and Hoeksema 2003, Hunt-Joshi et al. 2004, Staley et al. 2007, Wurst and van der Putten 2007). Comprehensive investigations on the interactions between three or more trophic levels above- and belowground are thus far lacking, although plant–herbivore interactions can be better understood when incorporating the third trophic level (Price et al. 1980, Vet and Dicke 1992). Progress in the study of above-belowground multitrophic interactions has been hampered by their inherent complexity (De Deyn and Van der Putten 2005) and their profound context dependency (Wardle et al. 2004).

The great majority of above- and belowground interactions are mediated by plants. The allocation of primary metabolites to above- or belowground tissues makes plants attractive resources for above- and belowground herbivores which themselves provide resources for higher trophic

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levels. To reduce or prevent herbivore damage, plants are armed with direct and indirect defenses. These defenses can be constitutive or inducible (Karban and Baldwin 1997). Direct chemical or physical defenses lower the plant quality for herbivores, or repel them entirely. Indirect defenses such as above- or belowground release of chemical compounds attract the natural enemies of the herbivores (the third trophic level) adversely affecting or killing the herbivores. Attack by fourth trophic level organisms may reduce the efficiency of natural enemies in controlling the herbivores. However, empirical data on trophic interactions up to the fourth or higher trophic levels is mostly restricted to aboveground systems (Harvey et al. 2003). Besides the indirect interactions between the plant and the third and higher trophic levels, plants and herbivores also indirectly interact with decomposers (Scheu 2001). Plants provide above- and belowground detritus input to the soil, and decomposers subsequently mineralise the majority of the detritus to plant-available nutrients. This mix of direct and indirect interactions makes above–belowground multitrophic interactions complex and, due to logistical constraints, barely accessible to empirical approaches.

The aim of our study was to investigate the impact of multiple trophic levels above- and belowground on plant and herbivore performance. Using a modelling approach we tested the hypotheses (1) that third and higher trophic level organisms influence (1a) plant performance and (1b) herbivore performance although the interactions with the plant are indirect, (2) that decomposers are vital components of networks of above- and belowground interactions affecting plant performance, and (3) that the effects of third and higher trophic levels and decomposers as tested in hypotheses (1) and (2) cross the soil–surface boundary.

We addressed the complexity of these hypotheses by developing a modelling framework for virtual experiments (sensu Peck 2004) that expands an existing greenhouse experimental system (Soler et al. 2005). In the model, we added trophic complexity and a full factorial design to the empirical system. We adopted this novel approach because it can cope with the complexity of multitrophic interactions without suffering from logistical or replication constraints. Additionally, our framework for virtual experiments retains a strong link to reality, because the model structure is based on an empirical study, the model parameters have been derived from empirical data from a variety of sources, and the model was validated against independent empirical data.

## Methods

### Model description

We developed a rule- and individual-based simulation model as a framework for simulation experiments that link ABove–BElowground multitrophic interactions (the ABBE model). We parameterized ABBE based on the species that Soler et al. (2005) used in their experimental system (Fig. 1). We simulated a system where the wild cruciferous plant *Brassica nigra* (Brassicaceae) is exposed to above- and belowground herbivory by the specialist chewing larvae of *Pieris brassicae* (Lepidoptera: Pieridae) and the specialized root-feeding larvae of the cabbage root fly *Delia radicum* (Diptera: Anthomyiidae), respectively. Aboveground, the third and fourth trophic levels are represented by the fairly specialized gregarious koinobiont endoparasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) and its secondary hyperparasitoid, the solitary idiobiont *Lysibia nana* (Hymenoptera: Ichneumonidae). As belowground third trophic level, we added the coleopteran egg predator *Aleochara billineata* (Coleoptera: Staphylinidae) to the setup of Soler et al. (2005).

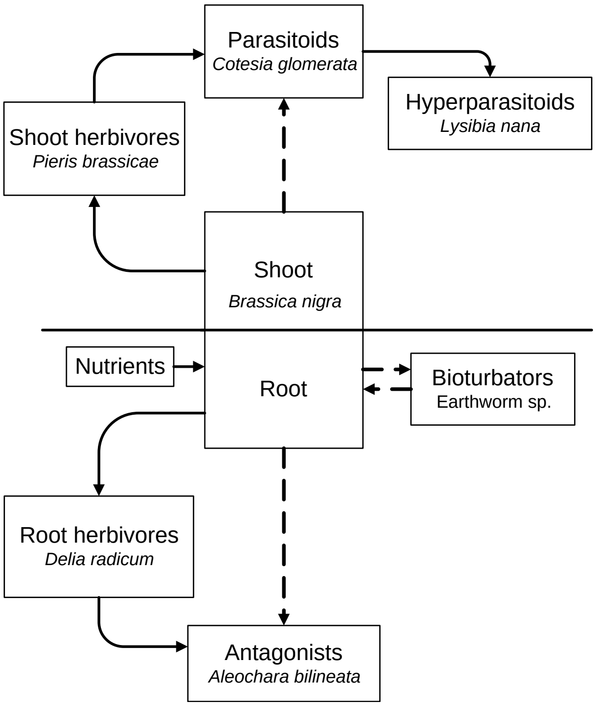

Figure 1. Overview of the above- and belowground trophic levels (boxes), their relationships (arrows), and the species representing the trophic levels (italics) in the simulation model ABBE. The central plant has a root and a shoot compartment that are divided by the soil surface. Relationships can be trophic (solid arrows) or non-trophic (broken arrows). Bioturbators were parameterized with data from the species *Aporrectodea caliginosa* and *Octolasion tyrtaeum.*

Our model description follows the overview – design concepts – details-protocol (ODD, Grimm et al. 2006) that aims at standardizing the description of individual-based simulation models. In the following Overview section, we provide a description of the essential model features. The complementary model details are provided in the Design concepts and Details sections in the Supplementary material Appendix 1. The model was implemented in the programming language C++ assisted by the software package Borland Builder 6.0. The executable files of the model are available upon request from the corresponding author.

### Overview

#### *Purpose*

The purpose of ABBE is to provide a framework in which simulated experiments can determine the impact of higher above- and belowground trophic levels for plant performance in a full factorial design.

### State variables and scales

To allow a comprehensive assessment of plant performance, the main experimental response variables in ABBE are the state variables plant root and shoot biomass and root and shoot quality for herbivores. To also assess the performance of second and higher trophic levels, additional state variables are numbers and body size of aboveground herbivores, parasitoids and hyperparasitoids, and numbers of belowground herbivores and their predators. To mimic typical greenhouse conditions and keep the model relatively simple, each trophic level is represented by one species (Fig. 1). For the same reasons, we model one single plant individual at the centre of above- and belowground interactions. To our knowledge, the available literature indicates the importance of individual-level variation such as phenotypic plasticity in body size only for the life cycles of aboveground species (Kaneko and Katagiri 2004). Therefore, we model aboveground species on an individual-by-individual basis with two life history stages each (premature and adult), while we consider belowground species at the level of populations.

Space is not considered explicitly because the effect of multitrophic interactions on plant performance can be evaluated without explicit reference to spatial relationships. The time step is six weeks and represents one average insect generation (see also Lemasurier 1991), except for belowground antagonists where the time step is 12 weeks (Fournet et al. 2000). The temporal extent is one growing season, here defined as 24 weeks, containing four or two time steps, respectively. The simulations start with plant seedlings that are 45 days old to match the conditions in Meyer (2000) whose data were used to parameterize initial biomass and nutrient conversion values (cf. Table A1 in the Supplementary material Appendix 1).

### Process overview

For all above- and belowground trophic levels, the processes of reproduction and mortality include demographic stochasticity to add realism to life cycle dynamics (see Supplementary material Appendix 1 for other sources of stochasticity and individual variation such as environmental stochasticity and phenotypic plasticity of aboveground trophic levels). For the plant, we explicitly model the processes of growth and mortality but not reproduction because the time frame of ABBE is one growing season and, therefore, the fate of the plant's offspring is irrelevant. Shoot and root growth depend on nutrient supply and root biomass, and mortality is solely caused by excessive herbivory assuming that other mortality sources are of relatively minor importance.

Aboveground trophic interactions include shoot herbivores reducing shoot biomass, parasitoids parasitizing shoot herbivores, and hyperparasitoids parasitizing parasitoids. Herbivore efficiency depends on parasitism (J. A. Harvey pers. comm.), while parasitoid efficiency is independent of parasitism by hyperparasitoids (J. A. Harvey pers. comm.). In every case, parasitism imposes an extra mortality on the hosts. Shoot herbivore presence increases shoot quality (Soler et al. 2005) and induces shoot volatile emission increasing parasitism efficiency (Ibrahim et al. 2005). We assume that the individual body size of a shoot herbivore depends on shoot quality while individual parasitoid and hyperparasitoid body sizes depend on their host's body size. Shoot herbivore, parasitoid and hyperparasitoid body size determine their egg load (Gilbert 1984, Lemasurier 1991, J. A. Harvey pers. comm.).

Belowground, trophic interactions between root herbivores and their predators result in an extra mortality of root herbivores due to predation. We assume that egg load of belowground predators depends on predation success. The surviving root herbivores reduce root biomass and quality as well as shoot quality (Soler et al. 2005, van Dam and Raaijmakers 2006). We include the mutualistic effect of earthworms representing the decomposers (Scheu and Falca 2000) and more specifically the bioturbators by increasing root biomass and quality as well as shoot quality in the presence of earthworms (Wurst and Jones 2003, Wurst et al. 2004, 2006) and leaving these parameters unchanged in their absence.

The sequence of the processes in the model is entirely motivated by the biology of the processes. For instance, predation mortality of root herbivores occurs before root herbivore feeding because the antagonists of the root herbivores are egg predators. On the other hand, parasitism mortality of shoot herbivores occurs after feeding because parasitized shoot herbivores usually do not immediately die and will have modified consumption patterns.

## Parameters and validation

The model structure of ABBE matches the experimental design in Soler et al. (2005) to allow model validation against their results. To keep the validation data independent, we excluded biomass data from Soler et al. (2005) from the calibration of model parameters. The set of most realistic parameter values (henceforth the standard parameter set) was to a large extent based on published and unpublished data combined with a few educated guesses where neither literature nor colleagues were able to provide estimates from empirical data (cf. Table A1 for the standard parameter set).

For model validation, we performed 1000 simulation runs of the model with the standard parameter set yielding four validation scenarios: shoot and root biomass of the plant both in the absence of any herbivory and in the presence of root herbivory. The effect of bioturbators was excluded from all validation scenarios and initial population size in the root herbivory scenario was adapted to match experimental conditions. We used biomass values from all four time steps of the model when the simulated plant was 87, 129, 171 and 213 days old and compared them to empirical biomass values from (Soler et al. 2005). Since the empirical biomass harvesting date at a plant age of 62 days was too early to be captured by the first time step of our model, we standardized simulated and empirically determined biomass values by dividing them by the number of days the respective plants grew. With a Mann–Whitney U-test, we compared the medians of the simulated biomass with the medians of the biomass of the eight replicates in the greenhouse experiment of (Soler et al. 2005).

To assess whether the stochasticity in the model generated realistic levels of variation, we compared the variance of the simulated and observed biomass values with F-tests separately for the four validation scenarios. To ensure equal sample sizes, we drew eight biomass values at random from the simulated biomass values and compared the variance of this subsample to the variance of the corresponding eight empirical replicates. We followed this procedure for 1000 subsamples and determined the proportion of non-significant comparisons.

## Simulated experiments

We designed a factorial experiment with presence and absence of aboveground herbivores, parasitoids, hyperparasitoids, and belowground herbivores, predators, and bioturbators as treatments (cf. Fig. A2 in the Supplementary material Appendix 1). We tested the effects of all ecologically relevant treatment combinations on the response variables shoot biomass, root biomass, plant mortality, and shoot and root herbivore mortality with 1000 simulation runs. A treatment combination is ecologically relevant when there are no higher trophic levels present without the underlying trophic level(s) present. We determined plant and herbivore mortality as the proportion of runs in which the plant or the herbivore, respectively, did not survive until the end of the growing season. Biomass of plants that did not survive until the end of the growing season was not included in the biomass assessment. In total, this amounts to 24 experimental scenarios and 24 000 simulation runs. For each experimental scenario, the standard parameter set was modified according to the treatment combination (e.g. initial shoot herbivore population size = 0 if shoot herbivores were absent) and then used as input for the simulated experiments.

We used the non-parametric Mann–Whitney U-test to analyse the effect of the treatments on the response variables root and shoot biomass at the end of the growing season because transformations did not remove non-normality of errors. We tested the effect of all six factors (aboveground herbivores, parasitoids, hyperparasitoids and belowground herbivores, antagonists, bioturbators) separately.

To test the effect of our treatments on the response variables plant mortality and aboveground and belowground herbivore mortality, we calculated the mortalities as the proportion of replicate runs in which the plant or the herbivore population did not survive until the end of the season. Note that we refer here to the mortality of the whole herbivore population that is emerging from the model at the end of the growing season and not to the mortality of individual herbivores that we use as input parameters. This calculation of the emerging mortality resulted in only one data point per treatment combination. Therefore, we used classification trees (Breimann et al. 1984) to classify and rank the trophic levels with respect to their effect on plant mortality and root and shoot herbivore mortality. For each of the three mortalities, we constructed one classification tree: The data set with the mortality values was successively split according to presence and absence of trophic levels by binary recursive partitioning so that the impurity within the remaining data sets was minimized. To avoid overfitting by allowing too many successive splits, we first determined the steeply decreasing curve of cumulative deviance explained by the tree with a certain number of splits for increasing numbers of splits. Then, for the final analysis, we limited tree size to that number of splits that initiated the asymptotic part of the curve where a further increase of the number of splits did not lead to the explanation of substantially greater amounts of deviance. The resulting tree sizes were 5, 4 and 3 splits for plant, aboveground herbivore, and belowground herbivore mortality, respectively.

## Sensitivity analysis

To assess the relative importance of the input parameters for model outputs such as root and shoot biomass, we performed a global sensitivity analysis with latin hypercube sampling to save processing time while covering as much parameter space as possible (McKay 1992). The relative importance of parameters can be used to rank the corresponding ecological processes with respect to their influence on the output. For all but the technical parameters of the model, 20 values covering the whole range of the parameter were specified and the order of the values was randomized for each parameter. The number of values that we used is a result of the tradeoff between maximizing the resolution of each parameter and minimizing simulation time. We chose the ranges and distributions of the parameter values in the sensitivity analysis according to best knowledge or field data distributions if available (Table 1). To minimize the risk of overfitting in the subsequent multiple regression of biomass versus the input parameters, as a rule of thumb at least 30 data points per parameter are required (Crawley 2007). For the 67 model input parameters in our sensitivity analysis, this results in a required minimum of 2010 data points. Hence, we repeated the specification of the 20 values per parameter 110 times yielding 2200 sensitivity analysis replicates of the parameter set. Each sensitivity analysis replicate was entered as input to ABBE generating root and shoot biomass values as outputs from 1000 simulation runs per sensitivity analysis replicate. After a successful check of normality and independence of errors supporting linearity, we evaluated the relationship between root or shoot biomass and the model input parameters with a multiple linear regression model. In light of the great number of parameters involved, we did not consider interactions in the regression model to keep it as complex as necessary and as simple as possible. This reduces the explanatory power of the model, but not its reliability. The regression models for root and shoot biomass were simplified by stepwise removal of the least significant parameters until the models contained only significant parameters ($p < 0.05$). Multicollinearity does not affect the results for two reasons. First, since the parameter value combinations to be tested in the sensitivity analysis were not measured, but specified by us, we were able to deliberately rule out correlation among parameter values by choosing parameter value combinations at random that spanned as much parameter space as possible. Second, we have devised the set of model parameters so that there are no interdependences among them by excluding model parameters from the calculations of derived model parameter values. This was the most parsimonious

Table 1. Results of the sensitivity analysis of ABBE. Significant parameters in a multiple regression of all ABBE model input parameters as explanatory variables versus the model outputs shoot and root biomass as response variables after model simplification. The greater the standardized regression coefficient, the greater is the importance of this model input parameter for determining the model output.

| Model input parameter | Standardized regression coefficient[1] | |
|---|---|---|
| | Shoot biomass | Root biomass |
| Annual nutrient supply rate | 9.46*** | 16.30*** |
| Nutrient conversion efficiency shoot | 6.98*** | – |
| Average initial body mass AG herbivores | 5.16*** | – |
| Proportional bioturbator effect on shoot mass | 5.07*** | – |
| Initial shoot mass | 4.64*** | – |
| AG parasitoid body length into mass[2] | 3.48*** | 2.54* |
| Initial root biomass | 2.86** | – |
| Natural mortality of BG herbivores | 2.84** | – |
| SD of initial AG herbivore body mass | 2.73** | – |
| Predation mortality of eggs | 2.65** | – |
| Initial number of BG herbivores | 2.63* | – |
| Number of eggs per AG herbivore body mass[3] | 2.58* | – |
| Initial number of BG antagonists | 2.54* | – |
| BG herbivore eggs killed by antagonist couple[4] | 2.50* | – |
| AG Hyperparasitism success probability | 2.45* | – |
| Natural mortality of AG parasitoids | 2.34* | – |
| AG herbivore low quality body mass[5] | 2.15* | – |
| Nutrient conversion efficiency root | – | 16.30*** |
| Nutrient uptake efficiency of root | – | 4.51*** |
| Natural mortality of AG hyperparasitoids | – | 3.95*** |
| Proportional bioturbator effect on root mass | – | 3.32** |
| Proportion of female AG parasitoids | – | 2.96** |
| Parasitism success probability | – | 2.87** |
| AG parasitoid head width into body length[6] | – | 2.83** |
| Natural mortality of AG herbivores | – | 2.32* |
| Proportional bioturbator effect on shoot quality | – | 2.26* |

AG – aboveground; BG – belowground; SD – standard deviation. [1]Standardized regression coefficients were calculated as regression coefficients divided by the respective standard error. [2]Exponent converting AG parasitoid body length into body mass. [3]Slope of the relationship AG herbivore number of eggs versus body mass. [4]Maximum number of BG herbivore eggs killed per antagonist couple lifetime. [5]AG herbivore body mass fraction on low quality plants. [6]Factor converting AG parasitoid head width into body length. ***p <0.001; **p <0.01; *p <0.05.

assumption, because we did not have any information on the existence of ecological dependences between model parameters. The only exceptions are some standard deviations. When standard deviations are derived as a proportion of the mean, we always kept the respective mean parameter during model simplification. The relative importance of the parameters with respect to root or shoot biomass is represented by the rank of their standardized regression coefficients, i.e. the rank of the regression coefficients divided by the corresponding standard errors. All statistical analyses were carried out with the software package R ver. 2.5.1 (R Development Core Team 2007).

## Results

During model validation, the simulated biomass did not significantly differ from the observed data reported in (Soler et al. 2005) (always $p > 0.5$ in Fig. 2). Validation was successful for four different setups: root and shoot biomass in the absence and presence of root herbivory (Fig. 2a–d). The variance of simulated shoot biomass without herbivory, root biomass without herbivory, shoot biomass with root herbivory, and root biomass with root herbivory was not significantly different from the variance of the observed data in 99%, 87%, 98% and 99% of the samples, respectively.

In the simulated factorial experiments, root herbivores greatly decreased root and shoot biomass at the end of the growing season ($p < 0.001$) while bioturbators greatly increased root and shoot biomass ($p < 0.001$, Fig. 3). Aboveground herbivores and parasitoids had small, but significant effects only on root biomass ($p < 0.001$, Fig. 3c–d). Aboveground herbivores affected root biomass positively while parasitoids had a negative effect.

Plant, root and shoot herbivore mortality were most strongly determined by belowground trophic levels (see the roots of the classification trees in Fig. 4). The third trophic level (aboveground parasitoids and belowground

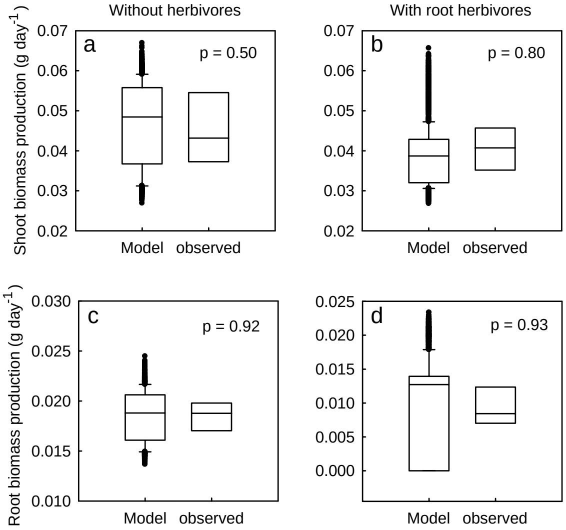

Figure 2. Model validation against observed data from (Soler et al. 2005). Observations included shoot (a, b) and root biomass production (c, d) without any herbivores (a, c) and with root herbivores (20 *Delia radicum* larvae per individual plant; b, d) over eight replicates each (observed). Simulations comprised 1000 replicate runs of ABBE with the standard input parameter values (model). Biomass data were converted to daily values to allow the comparison of empirical data with data from all four time steps in a simulated year. The modelled and observed plant biomass did not differ significantly in Mann–Whitney U-tests. Note the difference in the scales of the root and shoot biomass axes. In the boxplots, boxes contain the central 50% of the data (interquartile range) and are split by a line indicating the median. Whiskers indicate 1.5 times the interquartile range which corresponds to roughly two standard deviations. Individual points can be considered as outliers. Asymmetry of boxes indicates skewed data.

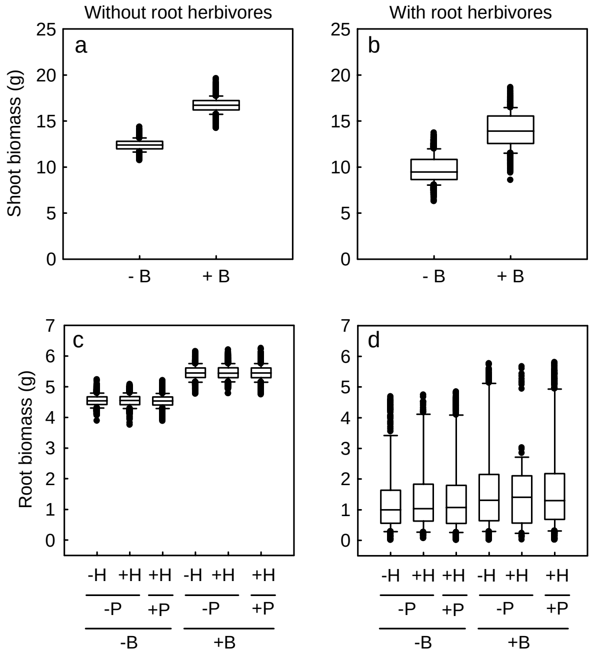

Figure 3. The effects of root herbivores, shoot herbivores, aboveground parasitoids, and bioturbators on shoot (a, b) and root (c, d) biomass as recorded for surviving plants at the end of a growing season for 1000 replicate runs of the simulation model ABBE. All effects shown here were significant at $p < 0.05$ in Mann–Whitney U-tests of presence versus absence of the respective trophic level (absence of root herbivores (a, c), bioturbators (−B), parasitoids (−P), and shoot herbivores (−H) and presence of root herbivores (b, d), bioturbators (+B), parasitoids (+P), and shoot herbivores (+H)). The combination −H+P does not exist because parasitoids cannot exist without herbivores. Please refer to the legend of Fig. 2 for an explanation of the boxplots.

antagonists) were either the most important trophic level (Fig. 4c) or the second most important one (Fig. 4a–b).

The strongest determinants of plant mortality in our simulations were the root herbivores (Fig. 4a). As expected, all plants survived when root and shoot herbivores were absent (seventh bar in Fig. 4a). Almost all plants survived when root herbivores were absent and shoot herbivores and their parasitoids were present (fifth bar in Fig. 4a). Plant mortality was 100% when root herbivores were present and not controlled by their antagonists (fourth bar in Fig. 4a) while aboveground, control of herbivores by parasitoids was less effective because parasitoid absence led to plant mortalities of less than 100% (second and sixth bar in Fig. 4a). Aboveground parasitoid control was stronger when root herbivores were absent than when they were present (compare the difference between fifth and sixth bar with the difference between first and second bar in Fig. 4a). Intermediate plant mortalities between 50% and 60% occurred when root herbivores were present and controlled by their antagonists and shoot herbivores were either present and controlled by their parasitoids or absent (first and third bar in Fig. 4a).

Shoot herbivores did not survive the growing season when root herbivores were present (first bar in Fig. 4b) or – trivially – when there were no shoot herbivores in the first place (last bar in Fig. 4b). When root herbivores were absent and aboveground parasitoids present, shoot herbivore mortality approached 100% (second bar in Fig. 4b). When aboveground parasitoids were not present to control shoot herbivores, then shoot herbivore mortality was greatly reduced (third and fourth bar in Fig. 4b). This effect was enhanced by the presence of bioturbators (third bar in Fig. 4b).

Root herbivores only survived when their antagonists were present (last bar in Fig. 4c showing 100% mortality). Root herbivore mortality was lowest when their antagonists controlled them and shoot herbivores were either not there or controlled by their parasitoids (first and thirs bar in Fig. 4c). If shoot herbivores were not controlled by their parasitoids, root herbivore mortality was 81% (second bar in Fig. 4c).

With the sensitivity analysis, we explored the parameter space of our model beyond the standard parameterization by assessing the impact of deviations of the input parameters from their standard values on output variables. As a result of the sensitivity analysis, the minimum adequate model of biomass as explained by the ABBE input parameters included 17 and 11 significant input parameters for the outputs shoot and root biomass, respectively (Table 1). Shoot and root biomass were most sensitive to the nutrient supply rate (Table 1). In general, parameters associated to nutrient uptake had the greatest standardized regression coefficients for both root and shoot biomass expressing their strong influence on these output variables. Shoot and root biomass shared one other significant parameter, the exponent of the relationship between individual body length and body mass of aboveground herbivores (Table 1). For both root and shoot biomass, parameters related to bioturbators and to the fourth trophic level, the aboveground hyperparasitoids, were among the most influential parameters (Table 1). The parameters with a great impact on shoot biomass were predominantly associated to the second trophic levels including more aboveground than belowground herbivore parameters. Above- and belowground third trophic level parameters were also among the significant parameters for shoot mass sensitivity, but of minor importance (at the lower end of the ranked standardized regression coefficients in Table 1). Root mass was mainly sensitive to third trophic level parameters which were all associated to the aboveground parasitoids.

## Discussion

In our simulated experiments, we found considerable effects of above- and belowground herbivores on plant biomass and mortality illustrating the importance of direct interactions for plant performance. Our well-validated model framework also offered the unique opportunity to go one step further and investigate whether plants and herbivores are also affected by indirect interactions with above- and or belowground third trophic levels or decomposers. The results of our simulations supported all three hypotheses: aboveground parasitoids and belowground antagonists as representatives of the third trophic level significantly affected plant biomass and mortality (Fig. 3c–d, 4a; hypothesis 1a) and were crucial in determining herbivore mortality (Fig. 4b–c; hypothesis 1b). Decomposers had a

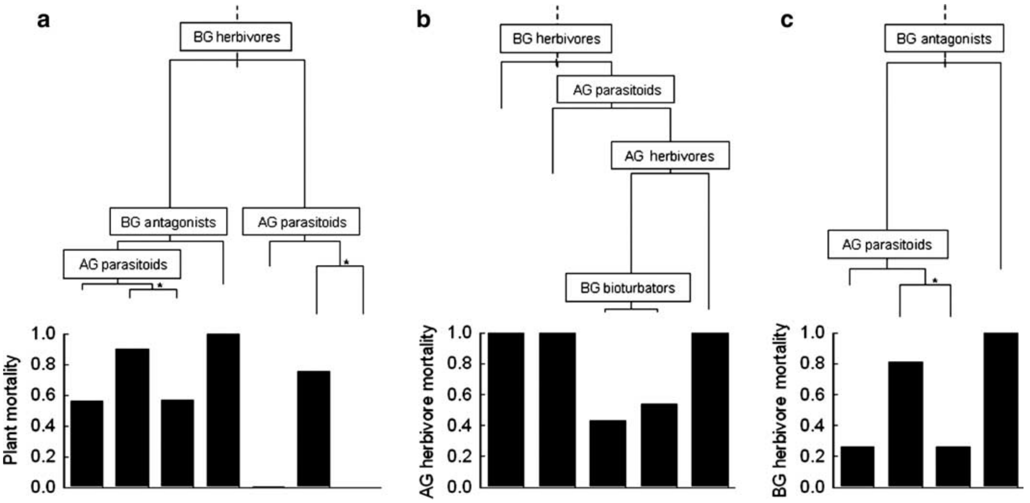

Figure 4. Classification trees for the effect of trophic levels (boxes) on plant mortality (a), aboveground (AG) herbivore mortality (b), and belowground (BG) herbivore mortality (c) in simulated experiments on all meaningful combinations of presence and absence of aboveground herbivores, parasitoids, and hyperparasitoids as well as belowground herbivores and their antagonists with 1000 replicate runs per treatment combination. Mortality was determined as the proportion of simulation runs that were not survived. Splitting the dataset according to presence (left branches) and absence (right branches) of the trophic level at the root of the classification tree (dashed line) explained most deviance in the data. The remaining dataset in each branch was split again according to presence/absence of that trophic level that makes the split explain most deviance in the remaining data. The relative length of the branches within each tree corresponds to the relative amount of deviance explained by the respective split. The bars in the lower part of the plot represent the mean mortality at the associated endpoint of the tree directly above each bar. *-AG herbivores.

substantial effect on shoot and root biomass and influenced herbivore mortality (Fig. 3a–d, 4b; hypothesis 2). The effects of the third trophic levels and the decomposers crossed the soil boundary corroborating interactions of above- and belowground organisms (Fig. 3c–d, 4a–b; hypothesis 3).

Our results support hypothesis 1a that the third trophic levels, the aboveground parasitoids and belowground antagonists, are of crucial importance for plant performance by strongly modifying the direct interactions between the herbivores and the plant. For example, the presence of belowground antagonists of root herbivores reduced plant mortality by up to 43%. Hence, the antagonists are powerful control agents of belowground herbivores thus underpinning the recently growing collection of case studies on efficient control of belowground herbivores by their natural enemies (Strong et al. 1996, Piskiewicz et al. 2007). The soil surface crossing effect of the interaction between third trophic levels and the plant (hypothesis 3) was apparent in aboveground parasitoids affecting belowground plant biomass and being more efficient in controlling shoot herbivores when root herbivores were present. These results agree with the few empirical studies on above- belowground interactions that have included the third trophic level (Bezemer et al. 2005, Soler et al. 2007a, 2007b).

The third trophic levels did not only play a crucial role for plant performance but also for the interaction between above- and belowground second trophic levels (hypothesis 1b). The interaction between above- and belowground herbivores was competitive because survival was only possible when the competitor on the other side of the soil surface was not present (Fig. 4b–4c) or controlled by their enemies at the third trophic level (Fig. 4c). Aboveground herbivore mortality only fell below 100% when neither belowground herbivores nor aboveground parasitoids were present (Fig. 4b). Hence, aboveground parasitoids and belowground herbivores interactively affected aboveground herbivore mortality. This above–belowground interaction is driven by the third and second trophic levels as equal partners in line with hypothesis 1b. The necessary condition for belowground herbivore population survival was the presence of their antagonists (Fig. 4c). This counterintuitive relationship can be explained by a boom-bust-behaviour of the root herbivore population: if the root herbivores were not controlled by their antagonists, they overexploited their resource and went extinct before the end of the growing season. The sufficient condition for less than 100% mortality of root herbivores was either the absence of shoot herbivores or their control by parasitoids (Fig. 4c). Thus, the belowground third trophic level had a strong direct stabilizing effect on the belowground herbivores while the aboveground third trophic level was a modifier of the competitive interaction between above- and belowground herbivores.

The third trophic level also influenced the symmetry of the competitive interaction between above- and belowground herbivores. Interspecific competition between herbivores is often asymmetric (Kaplan and Denno 2007), with most negative effects reported of aboveground on belowground herbivores and/or non-negative effects vice versa (Gange and Brown 1989, Moran and Whitham 1990, Masters and Brown 1992, Masters et al. 1993, 2001, Masters 1995, Soler et al. 2007c), and a few negative effects reported the other way round (Preus and Morrow 1999, Soler et al. 2005, Staley et al. 2007). In the present study, direct competitive effects between above- and belowground herbivores were symmetric, but indirect effects of the third trophic levels above- and belowground made the competitive relationship asymmetric. The aboveground parasitoids influenced belowground herbivore mortality while aboveground herbivore mortality was not affected by belowground antagonists.

Higher trophic levels beyond that of the herbivores have rarely been taken into account in above- belowground competition studies (for exceptions see Masters et al. 2001, Soler et al. 2005, Soler et al. 2007c), whereas our results highlight the importance of higher trophic levels for the symmetry of competitive relationships.

The fact that plant and herbivore mortalities of 100% occur in the simulation experiments may be more plausible when considering the time frame of the simulations. The mortalities emerging from the model are a cumulative measure of all deaths that have occurred until the end of the season, i.e. over a six months period. The duration of the majority of empirical studies of multitrophic systems may be too short to pick up such high mortalities – the study of Soler et al. (2005) covered two months. Nevertheless, studies supporting such high mortalities exist. For instance, Graham et al. (1986) report 100% parasitism mortality for the aboveground herbivore *Lygus hesperus* (Heteroptera: Miridae) on several weed species. Strong et al. (1995) link the complete die-off of the legume *Lupinus arboreus* (Fabaceae) to the presence of root-feeding caterpillars. In ABBE, the 100% mortality of the specialist shoot herbivores in the presence of root herbivores is an indirect effect. Uncontrolled root herbivores kill the plant causing starvation of all shoot herbivores by the end of the season. To further test the generality of these absolute predictions, we suggest experimental evaluation.

Our results also illustrate the importance of including indirect interactions with decomposers such as bioturbators in above–belowground interactions studies (Scheu 2001). Bioturbators were among the few treatments in our simulated experiment that had an effect on root and shoot biomass and they also reduced aboveground herbivore mortality when belowground herbivory and aboveground parasitoids were not present. These results are in line with the suggestions by Scheu (2001) that earthworms can be of much greater importance to plant performance than direct trophic interactions with herbivores. We restricted the earthworm interactions in our model to their effect on the plant, but earthworms can influence above–belowground interactions also by reducing root herbivore abundance (Blouin et al. 2005, Wurst et al. 2008). Our model offers a suitable framework for including this and other indirect effects in future extensions to test their relative importance for plant performance.

The sensitivity analysis allowed fine-tuning of the results from the absence–presence experiments because it reveals the impact of more gradual modifications of the interactions between trophic levels. Based on the number of significant parameters (Table 1), the majority of the sensitivity analysis outcomes reflect the conclusions from the removal experiment. However, two results are unique: First, shoot biomass was more sensitive to direct interactions with herbivores than to indirect interactions with higher trophic levels, while the opposite was true for root biomass. Second, shoot and root biomass were highly sensitive to hyperparasitoids, the fourth trophic level, – an effect that was not picked up in the simulated experiments. Presumably, the effect of the hyperparasitoids was too weak to perpetuate to the plant in a presence–absence experiment. In contrast, a sensitivity analysis focuses on gradual modifications and was therefore able to pick up the hyperparasitoid effect. Hence, the fourth trophic level can potentially affect the plant strongly, corroborating hypothesis 1a also for trophic levels higher than the third.

Overall, we found more effects of belowground trophic levels than aboveground trophic levels on plant performance. The majority of the studies that have addressed the relative importance of above–belowground interactions for plant performance report more significant aboveground than belowground effects on a variety of plant performance surrogates (Moran and Whitham 1990, Masters 1995, Müller-Schärer and Brown 1995, Hufbauer and Root 2002, Rudgers and Hoeksema 2003, Hunt-Joshi et al. 2004). In contrast, only a few studies support our results that belowground interactions are more important for plant performance than aboveground interactions (Gerber et al. 2007, Wurst and van der Putten 2007).

Our model framework is well suited to explore the determinants of above- versus belowground effects, because it can easily be parameterized and validated with data from other case studies. Currently, the model results are largely independent of the study of Soler et al. (2005) because they share only the species identities and a few input parameter values. More than 90% of the model parameters have been derived from independent sources as indicated by the references. By developing an individual-based model, we adopted a bottom-up approach where community-level effects emerge from individual life histories and interactions. Hence, the model results are not imposed by the effects observed in the underlying empirical study. In future applications, our model can be more efficient than empirical approaches due to its independence of logistical constraints. For instance, the complexity (24 treatment combinations) and replication (1000 runs) of the present study correspond to an experiment with 24 000 pots in the greenhouse. However, a simulated experiment depends on empirical experiments for conceptualization, parameterization, and validation. Particularly, a thorough validation against multiple observed patterns as performed in this study is crucial (Grimm et al. 2005). The generality of the conclusions that can be drawn from a simulated experiment is limited to almost the same extent as it is for the underlying empirical experiments. Only by exploring the parameter space of the model during the sensitivity analysis, modelling approaches offer an opportunity for extending the generality of the conclusions to some extent. However, by including new data, future model extensions of ABBE will be highly suitable to test the context-dependency of aboveground and belowground multitrophic interactions (Wardle et al. 2004).

We conclude from our simulated experiments that the consideration of above- and belowground third and higher trophic levels as well as decomposers can change the outcome of above–belowground bitrophic interactions considerably and that this effect crosses the soil–surface boundary. Based on the expansion of an empirical experiment to greater trophic complexity, we were able to more completely analyse above- and belowground effects of trophic levels and decomposers on plant and herbivore performance in this system, enhancing the generality of our conclusions. Our results emphasize the importance and the benefits of integrating empirical and modelling approaches.

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Supplementary material (available online as Appendix O17220 at www.oikos.ekol.lu.se).