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Scaling up phenotypic plasticity with hierarchical population models

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Abstract

Individuals respond to different environments by developing different phenotypes, which is generally seen as a mechanism through which individuals can buffer adverse environmental conditions and increase their fitness. To understand the consequences of phenotypic plasticity it is necessary to study how changing a particular trait of an individual affects either its survival, growth, reproduction or a combination of these demographic vital rates (i.e fitness components). Integrating vital rate changes due to phenotypic plasticity into models of population dynamics allows detailed study of how phenotypic changes scale up to higher levels of integration and forms an excellent tool to distinguish those plastic trait changes that really matter at the population level. A modeling approach also facilitates studying systems that are even more complex: traits and vital rates often co-vary or trade-off with other traits that may show plastic responses over environmental gradients.

Here we review recent developments in the literature on population models that attempt to include phenotypic plasticity with a range of evolutionary assumptions and modeling techniques. We present in detail a model framework in which environmental impacts on population dynamics can be followed analytically through direct and indirect pathways that importantly incorporate phenotypic plasticity, trait-trait and trait-vital rate relationships. We illustrate this framework with two case studies: the population-level consequences of phenotypic responses to nutrient enrichment of plant species occurring in nutrient-poor habitats and of responses to changes in flooding regimes due to climate change. We conclude with exciting prospects for further development of this framework: selection analyses, modeling advances and the inclusion of spatial dynamics by considering dispersal traits as well.

Introduction

Plants can adapt to variable environments by changing their phenotype which typically is expected to increase individual fitness (Pigliucci 2001; Sultan and Stearns 2005; Bradshaw 2006). Despite the expectation that phenotypic plasticity (i.e. environmentally induced trait variation) will have important consequences for population dynamics at the local and landscape scale (Sultan 2007), studies of phenotypic plasticity typically focus on individual fitness. The effect of phenotypic plasticity across environments on fitness components like reproduction or survival can be analyzed statistically with path models or structural equation models. Path

models fit hypothesized networks of causal relationships between ecological drivers, individual traits and one or more fitness components to data (Huber et al. 2004; Pigliucci and Kolodynska 2006; Picotte et al. 2007; de Vere et al. 2009). However, finding effects of phenotypic plasticity on a fitness component does not automatically allow for conclusions at the population level (Metcalf and Pavard 2007). The relationship between phenotypic plasticity and population dynamics is unlikely to be straightforward: phenotypic shifts in one trait may have indirect fitness consequences through positively or negatively (e.g. trade-offs) correlated traits (Tonsor and Scheiner 2007). Furthermore, changes in individual fitness rarely translate linearly into population size fluctuations (Ehrlén 2003), partly because not all fitness components are equally important for local population growth and partly because not all individuals will respond in the same way.

Evaluation of the population-level consequences of phenotypic plasticity requires computer simulations or, more elegantly, analytical population models (Caswell 1983). Matrix population models have proven to be very useful because they transparently represent the life cycle of a species by including all the year-to-year transitions between the various age or size stages in which individuals can be classified (Caswell 2001). These annual transitions are made up of vital rates (i.e. fitness components) such as stage-specific survival and reproduction rates and growth rates of surviving individuals that reach other stages. The mathematical characteristics of matrices have clear biological interpretations such as the projected population growth rate (i.e. the dominant eigenvalue of the transition matrix) and the relative contributions of matrix elements or vital rates to population growth (i.e. elasticity values) (de Kroon et al. 2000; Franco and Silvertown 2004). Matrix models have continued to develop rapidly and now include stochasticity (Tuljapurkar et al. 2003) and a spatial dimension (Neubert and Caswell 2000), while still retaining all useful analytical properties.

Matrix population models have already been used to investigate the consequences of the outcome of phenotypic plasticity, for instance reduced variability in demographic rates due to dampening of the impact of environmental fluctuations (Caswell 1983). Temporal variation in demography is generally thought to decrease population growth (Tuljapurkar 1990; Boyce et al. 2006), although that still depends on the specific response (e.g. linear or convex) of a vital rate to an environmental driver (Koons et al. 2009). It has therefore been hypothesized that natural selection has led to the reduction of the variation of especially those vital rates that contribute most to the population growth rate (Pfister 1998; Morris and Doak 2004). However, these studies did not specifically include the plastic traits that may underlie vital rate variability.

In this paper, we develop a framework of hierarchical population models (HPMs) to analyze the effects of phenotypic plasticity on demographic and dispersal traits at the population level. In this context we will investigate plastic changes of morphology, biomass accumulation, flowering probability and reproductive effort; traits are directly and indirectly linked with demography and dispersal processes. In essence, HPMs bring together two research lines: that of studying the effects of phenotypic plasticity with path models and that of spatial and non-spatial population modeling. This approach of coupling relationships between individual traits and vital rates inside matrix models was already pioneered by van Tienderen (2000) with an hypothetical plant species, and applied to and extended for animal field data by Coulson and coworkers (Coulson et al. 2003; Coulson et al. 2006; Pelletier et al. 2007; Coulson and Tuljapurkar 2008). Here we develop HPMs for perennial plants and add spatial dynamics to the equation. We will illustrate how HPMs can be used to answer the following important questions: what are the

population-level consequences of trait-trait covariance and how does phenotypic plasticity change the effect of environmental fluctuations on local and spatial population dynamics.

Hierarchical population models

HPMs can be schematically represented (as for instance in Fig. 1) in the same way as path models: environmental factors (i.e. ecological drivers such as flooding, nutrient availability, weather, or population density) influence traits of individuals, which in turn affect vital rates (or fitness components such as survival, growth and reproduction) that together can be used to build population models (e.g. a population transition matrix). Thus, each of these lower-level parameters (i.e. environmental factors, traits of individuals, vital rates) can influence population dynamics. Vice versa (from right to left in Fig. 1) the arrows leading to a model parameter indicate which lower-level parameters contribute to that higher-level parameter. Like in path models covariances between individual traits can be included in HPMs. Trade-offs among traits result in negative covariances. Depending on how individual traits of interest are defined, HPMs may also include direct effects of environmental factors on vital rates ('v' in Fig. 1). It is also possible that the changes in the environment affect how an individual trait contributes to a vital rate (i.e. the vital rate function of that trait changes with the environment).

However, to our knowledge no such complex hierarchical population models including the relationships described above (Fig. 1) have been performed so far. In the next section we will present the results of a case study (Fig. 2) for which we have data and for which we show numerically what insights can be gained from a HPM approach. Thereafter we will explore a more complex, hypothetical case study which includes various environmental effects and trait-trait covariation.

Eutrophication effects on the population dynamics of 4 grassland species

The first case study is an example of how an HPM can be constructed and analyzed. We analyzed the population-effect of eutrophication, which has caused declines in species richness in many grasslands (Neitzke 2001; Stevens et al. 2004). We focused on four perennial plant species (*Centaurea jacea*, *Cirsium dissectum*, *Hypochaeris radicata* and *Succisa pratensis*) of which the demography has been studied in nutrient-poor grasslands (Jongejans and de Kroon 2005; Jongejans et al. 2008).

To study the importance of lower-level parameters we formulated an HPM (see Fig. 1 for details) with the following plant traits: plant size (z_1), threshold size for flowering (z_2) and seed production per unit plant size (z_3). With z_1 we fit linear models to the following vital rates: the number of clonal offspring per non-flowering (w_4) and per flowering rosette (w_5) and the number of seeds produced per flowering rosette (w_9). The slope of the latter seed production model is the plant trait z_3, the number of seeds per unit plant size. For adult survival (w_2, w_3) and flowering (w_6, w_7, w_8) we performed generalized linear models with a logit-link and plant size as the explanatory variable. We inserted the average of observed plant sizes into these functions to obtain average vital rate values for the field scenario (see Supplementary Material for details).

The field scenario (i.e. control, nutrient-poor conditions) was contrasted with an eutrophication scenario, which was based on the field scenario, but altered at five points: the three plant traits (z_1, z_2, z_3), and two direct effects (v_1, v_2) on the vital rates survival and seedling establishment. For the changes in plant traits and survival we used the relative effects that were found in a garden experiment in which these four plant species were grown amidst a hexagon of tussocks of the competitive grass *Molinia caerulea* (Jongejans et al. 2006). Half of the plots in the garden were annually fertilized, and by comparing survival and the sizes of the survivors between the enriched and control plots we were able to estimate how much nutrient enrichment,

as applied in the garden experiment, affects the mentioned plant traits and the adult survival rate. For the calculation of the vital rates of the eutrophication scenario we changed the mean plant traits of the field scenario proportionally to the experimental fertilization effect sizes which can be found in Table 2 (see Supplementary Material for details). For the relative effect of eutrophication on seedling establishment (w_{10}) we used the ratio of the establishment rate in high productive field sites and the establishment ratio in low productive field sites as found in a published seed addition experiment involving 20 sites (Soons et al. 2005).

Next we wanted to know how these different effects of eutrophication on plant traits and vital rates contributed to the difference ($\Delta\lambda$) between the projected population growth rate of the eutrophication scenario (λ^E) and that of the default field scenario (λ^C). We therefore decomposed $\Delta\lambda$ with a so-called fixed-effect LTRE (i.e. Life Table Response Experiment; Horvitz et al. 1997; Caswell 2001; Jongejans and de Kroon 2005) to investigate at each level what caused the difference between λ^C and λ^E. LTREs approximate these contributions to $\Delta\lambda$ with the products of 1) the sensitivity of λ to changes in a parameter and 2) the deviation of the value of that parameter from its control value (see Supplementary Material for the sensitivity and LTRE equations used for the trait, vital rate and matrix element levels). LTRE contributions of underlying parameters quantify the importance of those parameters for the given difference in λ and together the contributions sum up to the total λ-difference observed. First we decomposed $\Delta\lambda$ at the level of the matrix elements (a_{ij}), then at the level of the underlying vital rates (w_k), and finally at the level of the involved plant traits (z_r). The last level also included the contributions of changes in direct environmental effects (v_h) on vital rates (see Fig. 1). This way the sum of the LTRE contributions at each level approximated $\Delta\lambda$.

Eutrophication had a larger impact on λ of the two shorter-lived species: $\Delta\lambda$ was -0.626 (from λ^C=0.960 to λ^E=0.334) for *Hypochaeris radicata* and -0.496 (from 1.007 to 0.511) for *Cirsium dissectum*, while only -0.059 (from λ^C=0.986 to λ^E=0.928) for *Centaurea jacea* and even +0.045 (from λ^C=1.237 to λ^E=1.282) for *Succisa pratensis*. Furthermore, the LTREs clearly showed that nutrient enrichment affected the population dynamics of these four grassland species differently (Fig. 2): at the level of matrix elements we see that the steep decline in λ in the eutrophication scenario for the short-lived species was mostly caused by decreased survival (and by decreased clonal propagation for *C. dissectum*). However, reduced sexual reproduction had the largest negative contributions to $\Delta\lambda$ in the two longer-lived species (*C. jacea* and *S. pratensis*), although this was more than compensated in *S. pratensis* by positive contributions of matrix elements that represented the fate of surviving individuals.

At a lower level we see that this negative contribution of sexual reproduction in the long-lived species is mainly caused by reduced seedling establishment (w_{10}). At this vital rate level it becomes clear that the largest buffering of the lower establishment rate in *S. pratensis* actually takes place within the sexual reproduction matrix elements by increased seed production (w_9). The vital rate analysis also shows that it is not the survival rate itself that contributed to a higher λ in the eutrophication scenario, but that this was caused by higher flowering probabilities of surviving plants (w_6, w_7). For the short-lived species it was mainly the reduction in the survival rate of non-flowering plants (w_2) that caused the λ-declines.

Direct effects (v_1 and v_2) of eutrophication had the largest negative contributions at the lowest level (see right column of Fig. 2). These negative effects on λ were to some extent buffered by positive contributions by changed plant traits, showing that plastic responses to eutrophication of plants that are growing amidst competitors can indeed be beneficial for

population growth. Increased plant size and increased seed production per unit biomass were especially important in *S. pratensis*, and less so in *H. radicata* and *C. jacea*. The last species, *C. dissectum*, did not show any of these plastic responses and it might well be that that contributes to the vulnerability of this declining Red list species (see also Jongejans et al. 2008; de Vere et al. 2009). Together these multi-level LTRE analyses showed which traits show plastic responses that are actually important for population growth and how the importance of phenotypic plasticity relates to direct environmental impacts. These analyses also showed which vital rates, and subsequently matrix elements, were affected most.

Phenotypic plasticity in response to flooding

In the second case study we explore a more complex situation. In disturbed habitats such as river floodplains, traits like root aerenchyma, anaerobic metabolism and rapid petiole elongation are essential for survival during summer floods (van der Sman et al. 1993; Mommer et al. 2006). Other traits like seed buoyancy and traits that affect the amount and timing of seed production (van Splunder et al. 1995; Boedeltje et al. 2004) also play crucial roles in the dynamics of plant populations. Variation in these plant traits among species in experiments has been shown to adequately explain the altitudinal zonation of plant species in floodplains (van Eck et al. 2004; Voesenek et al. 2004; Lenssen and de Kroon 2005; van Eck et al. 2006). Environmentally induced trait variation enhances individual fitness in variable environments, and is thus expected to be particularly important for preventing large changes in population size in flood-prone areas (Verschoor et al. 2004; Miner et al. 2005). However, a trade-off between adaptations to survival during flooding and investments in reproduction (van der Sman et al. 1993) makes the effect of flood-related survival traits on population growth complex. It is therefore very insightful to formulate these relationships for this flooding system in a hierarchical population model (Fig. 3). With an HPM the importance at the population level of direct and indirect pathways through environment-trait, trait-trait, and environment/trait-vital rate relationships can be disentangled.

For instance the plastic formation of root aerenchyma in some plant species (or genotypes of the same species) in response to partial flooding enables oxygen to diffuse to the roots of these species, thereby increasing their survival (Visser et al. 2000; Huber et al. 2009). For our modeling exercise it is important to realize that not only the plant trait is changing with the environment (phenotypic plasticity; '*p*' in Fig. 3), but also the dependency of the survival rate on the amount of aerenchyma in the roots ('*d*' in Fig. 3): aerenchyma is crucial for survival when submerged, but contributes little to survival under drained conditions. Additionally, flooding may also affect survival directly ('*v*' in Fig. 3), in a way that does not involve variation in any traits or through plant traits that are not included in the model.

Another plastic trait through which some plants can increase their survival when flooded is plant height, since elongated stems that reach the water surface can supply submerged plant parts with oxygen (Blom and Voesenek 1996; Pierik et al. 2009). On the other hand, constitutive plant height at which seeds are released during seed set is also a determinant in seed dispersal by wind. More research is needed to know if and how flooding induced and constitutive plant height are related ('*c*' in Fig. 3) (cf. Weijschedé et al. 2006). Since flooding itself can increase seed dispersal distances ('v' in Fig. 3; Blom and Voesenek 1996), it might be that flooding has both direct and indirect effects on dispersal. Water, wind and other dispersal vectors can be combined in so-called total dispersal kernels (Nathan 2007). Taking dispersal parameters into account may or may not matter for local population dynamics, but it is crucial when considering spatial population dynamics (Fig. 3).

Piecing all these relationships together might be challenging, but also very worthwhile. The HPM in Fig. 3 may for instance be applied to investigate how phenotypic plasticity allows some plant species to survive when flooding regimes change due to global climate change (e.g. shifted precipitation phenology and increased melting of glaciers). The approach outlined in Figure 3 can, with relatively small adaptations of the input parameters and the underlying relationships, be used to study the response of populations to variation in other ecological drivers such as CO_2, nutrient availability or salinity. Ultimately, HPMs can be used to test the promises of phenotypic plasticity as a mechanism for buffering effects of climate change, environmental stochasticity and habitat heterogeneity (Agrawal 2001; Callaway et al. 2003; Sultan 2007) by calculating the net effect of phenotypic plasticity at the population level.

Elderd and Doak (2006) compared the flooded and unflooded population dynamics of *Mimulus guttatus*, and found that of all the considered vital rates it was the increased germination rate and increased summer survival that caused the higher population growth in the flooded habitat. Similarly, Smith et al. (2005) found that population growth of the endangered *Boltonia decurrens* depends on the combination of early floods and precipitation. What HPMs could add to these interesting studies is to quantify how much plastic response of the involved traits contributed to population growth in flooded habitats and to quantify the selection pressures on these traits in flooded and unflooded scenarios. Spatial HPMs are especially promising for comparing the roles of local plastic responses and escaping adverse conditions by dispersal. Such comparisons are especially of interest in the context of climate change and range shifts. A research agenda for population studies in floodplains could therefore include the following research questions:

a) How does phenotypic plasticity contribute to buffering environmental fluctuations at the local population level, and which plant traits and vital rates are directly and indirectly involved in mediating these fluctuations?
b) To what extent can phenotypic plasticity buffer harsh environmental conditions in different landscape configurations, and how does it relate to the alternative strategy of escape by dispersal?
c) Which life histories enable population persistence under past, present and future scenarios of flooding regimes, and is restoration management needed to prevent species loss as the climate continues to change?

Obviously, these research questions not just apply to flooding systems but also to any ecosystem where climate change is an important ecological driver and where the habitat ranges of species shift as a result of environmental changes.

Discussion

The pioneering hierarchical population model (HPM) of van Tienderen (2000) shows how traits like seed mass, germination time and flowering time shape the vital rates of a hypothetical annual plant. He quantified the direct effects of a trait on population growth via various vital rates and matrix elements as well as the indirect effects through correlations with other traits. Our first case study, in which we constructed a HPM with field and experimental data (Figs. 1 and 2), suggests that the potential of phenotypic plasticity to buffer detrimental environmental changes is species-dependent. In some species (e.g. *S. pratensis*) directly negative environmental impacts on survival seemed to have been buffered by increased plant size and sexual biomass allocation, whereas in other species (e.g. *C. dissectum*) such buffering by plastic responses was mostly absent. These exploratory studies suggest that HPM can indeed be very insightful for

studying the importance of phenotypic plasticity for population dynamics under changing conditions.

Studying phenotypic plasticity with HPMs

In the eutrophication case study the incorporated traits were plant size and two traits that shape the amount of seed production (i.e. the threshold size for flowering and the number of seeds produced per unit plant size). In studies on phenotypic plasticity such biomass-related traits are often considered to show 'passive' phenotypic plasticity because the change in plant size in response to nutrient enrichment may simply be a consequence of a direct relationship between resource availability and biomass accumulation which is not controlled by changes in developmental processes (Sultan 1995; van Kleunen and Fischer 2005; Kurashige and Callahan 2007). On the other hand, morphological changes such as stem elongation or aerenchyma formation in response to ecological drivers (Fig. 3) are seen as more 'active' phenotypic plasticity.

However, this distinction between active and passive phenotypic plasticity are not always easy to make. For instance, an increase in leaf size under shaded conditions can be considered an active foraging response (sensu Hutchings and de Kroon 1994) if it increases resource uptake compared to a non-plastic genotype. Shade induced reduction of leaf elongation, on the other hand, can be considered as reflecting a resource driven passive response (van Kleunen and Fischer 2005, 2007). A reduced leaf size under shaded conditions may also reflect an active response if it conserves resources and increases survival relative to a genotype producing larger leaves. The distinction between active and plastic responses can be of interest when comparing the genetic and physiological regulations of phenotypic plasticity at various stages of an individual's development. However, as shown in Figs.1 and 3, different types of traits and responses (e.g. morphological, biomass, ratios) can be included in HPMs in similar ways. More important is the functionality of traits when deciding to incorporate them in HPMs: how well does a trait determine vital rates that are crucial parts of the life cycle, and thereby has the potential to significantly influence population dynamics.

HPMs are well-suited to study other important aspects of phenotypic plasticity as well. By comparing similar models that differ only in one or two key functions, the importance of including various degrees of phenotypic plasticity can be evaluated at the population level. The same methodology can be used to see if any costs of phenotypic plasticity, in terms of reduced growth and reproduction or costs through trade-offs with other traits, are actually of significance. A wide variety of environmental conditions can be fed into stochastic Monte Carlo simulations to investigate to what extent phenotypic plasticity can mediate local and regional population persistence under variable conditions. Stochastic elasticities (Tuljapurkar et al. 2003) can then be used to analytically examine the simulation results and to see which model components contribute most to the population growth rates.

Another promising avenue in further developing HPMs is to incorporate continuous trait variables and plastic responses over the range of trait values, instead of merely studying changes in trait means. This could be achieved by adopting the methodology of integral projection models, which are similar to projection matrices but have continuous rather than discrete stage variables (Easterling et al. 2000; Rees and Rose 2002; Ellner and Rees 2006) and are therefore especially useful for studying the population consequences of environment-trait-life history relationships and their variances.

Studying selection gradients with HPM

van Tienderen (2000) calculated integrated elasticity values and selection gradients for individual traits while taking into account both the direct effects of a trait on population growth via various vital rates and matrix elements, and the indirect effects through correlations with other traits. One of the advantages of matrix population models for selection analysis is that an annual, integrated estimate of fitness can be distilled rather than a life-time fitness approach, which does not take variation in life span among individuals into account (Coulson et al. 2006). Rees and Rose (2002) analyzed the selection pressure on the threshold size for flowering in a population of the monocarpic perennial *Oenothera glazioviana*, although they did not include any ecological driver or phenotypic plasticity. By changing input parameters (e.g. increasing or decreasing leaf size) one can test the effects of these changes not only on survival or seed output of plants, but also on growth of the whole population. If, for instance, reduction in biomass is associated to increased survival it may still ultimately result in positive (or less negative) population growth rates. Or it may not always be beneficial to increase seed set at the cost of survival if there are too few safe sites to ensure seedling establishment.

Hierarchical population models have successfully been developed and applied to detailed, long-term field data on red deer (Coulson et al. 2003) and soay sheep (Pelletier et al. 2007). The selection analyses in these studies are based on λ-sensitivity values (Coulson et al. 2003; sensitivity values also form the basis of the LTRE approach in Fig. 2), in contrast to a focus on proportional responses (i.e. elasticities, van Tienderen 2000). These studies sophisticatedly show that traits like birth weight influence population growth through different vital rates, and that the contributions of these different pathways fluctuate strongly from year to year. Interestingly, these survival rates of different ages and genders responded differently to environmental drivers, resulting in no overall correlation between environmental fluctuation and selection (Coulson et al. 2003). Furthermore, the effect of positive selection on birth weight was buffered by the fact that birth weights of offspring tended to be smaller than those of the parents (Coulson and Tuljapurkar 2008).

HPMs are thus an excellent method to study trait selection and microevolution, because these models consider the role of a trait within the context of the entire life cycle and a set of ecological interactions (Metcalf and Pavard 2007; Knight et al. 2008). This would address one of the weaknesses of most studies on phenotypic plasticity where simply biomass or seed set is used as a fitness parameter to investigate which traits are under selection. Selection gradients may be very different for individual plants than for individuals that are part of a population and even more so for individuals in populations that are immersed in a community of different species. Ultimately, the seed production of a single plant needs to be evaluated with respect to the total number of seeds in the population in combination with the proportional germination and survival of seedlings to reproducing plants, in order to determine what the contribution of that plant is to future generations. HPMs might also prove useful tools for unraveling the population impact of trait variation further, at the genetic level (Metcalf and Mitchell-Olds 2009).

Conclusions

The future of hierarchical population models looks bright as new techniques are currently being developed to link statistically advanced path models to models of population dynamics. Statistically sound structural equation models also form a good tool for quantifying explained and unexplained variance at each higher-level upstream parameter (see e.g. Bakker et al. 2009 for a sophisticated analysis of the extinction risk of Californian island foxes). Information on explained variance is very useful for introducing individual and environmental stochasticity (Fox and Kendall 2002) into these local and spatial population models to study the importance of

variation in individual traits due to environmental variation. As seen in this paper, HPMs bring together path analyses and population projection models, and form an excellent stage for studying the consequences of phenotypic plasticity for not only single fitness components, but importantly also for population dynamics.

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533 **Table 1**. Definition of all the stage classes and model parameters used in Figure 1. The C-index
534 indicates the control, field scenario. The *a*'s and *b*'s are empirically determined constants (see
535 Supplementary Material).

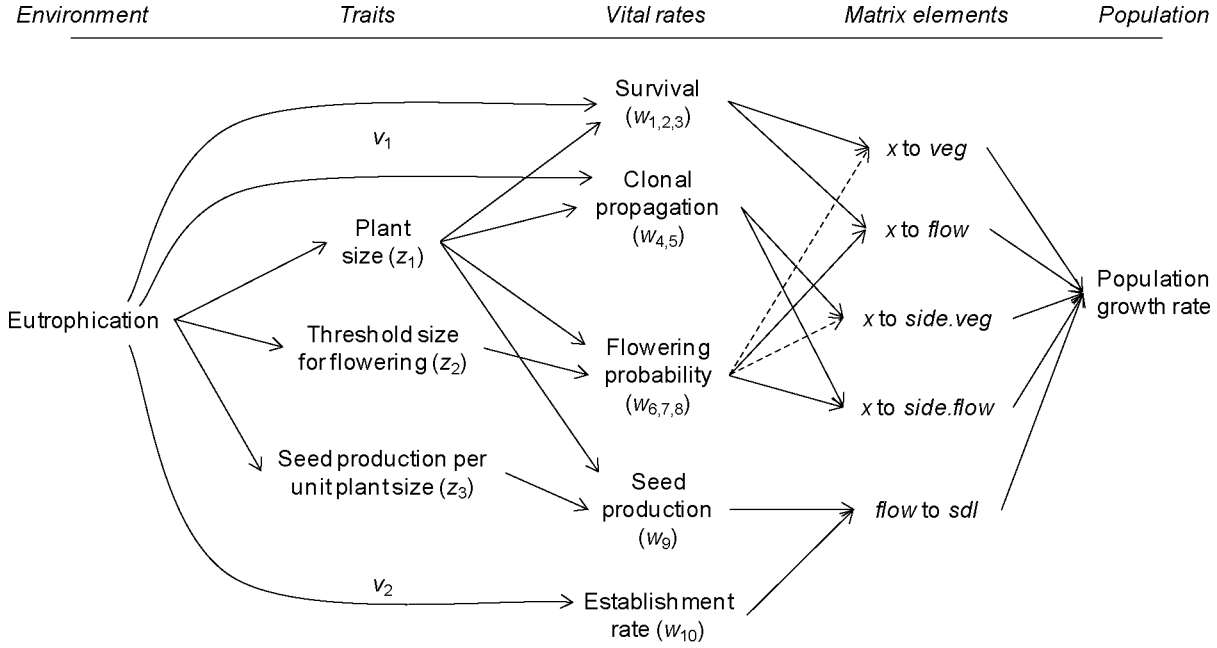
Param.	Definition
	The five stage classes of the matrix model are:
sdl	new seedlings
veg	non-flowering rosettes that are older than one year
flow	flowering rosettes that are older than one year
side.veg	new, non-flowering clonal offspring
side.flow	new, flowering clonal offspring

	The 10 vital rates (and their plant trait functions) are:
w_1	survival rate of *sdl*: $w_1 = v_1 w^{c}_1$
w_2	survival rate of *veg* and *side.veg*: $\text{logit}(w_2/v_1) = a_2 z_1 + b_2$
w_3	survival rate of *flow* and *side.flow*: $\text{logit}(w_3/v_1) = a_3 z_1 + b_3$
w_4	clonal propagation rate of *veg* and *side.veg*: $w_4 = v_1(a_4 z_1 + b_4)$
w_5	clonal propagation rate of *flow* and *side.flow*: $w_5 = v_1(a_5 z_1 + b_5)$
w_6	flowering probability of surviving *veg* and *side.veg*: $\text{logit}(w_6) = a_6(z_1 - z_2) + b_6$
w_7	flowering probability of surviving *flow* and *side.flow*: $\text{logit}(w_7) = a_7(z_1 - z_2) + b_7$
w_8	flowering probability of new *side.veg* and *side.flow*: $\text{logit}(w_8) = a_8(z_1 - z_2) + b_8$
w_9	seed production per *flow* and *side.flow*: $w_9 = z_3 z_1 + b_9$
w_{10}	*sdl* establishment rate per seed: $w_{10} = v_2 w^{c}_{10}$
	The 3 plant traits are defined as follows:
z_1	plant size: the product of the number of leaves and maximum leaf length
z_2	additional threshold size for flowering in the nutrient enriched situation ($z_2 = 0$ in the control situation)
z_3	seed production per unit plant size
	The 2 direct environmental effects on vital rates are:
v_1	effect of eutrophication on plant survival and clonal propagation (which also involves survival till the next year)
v_2	effect of eutrophication on seedling establishment

Table 2. The experimentally determined relative impacts of nutrient enrichment on different plant traits and vital rates. The control, field scenario values apply to all four studied grassland plant species.

Affected plant traits and vital rates	Control, field scenario	Eutrophication scenario			
		Cirsium dissectum	*Hypochaeris radicata*	*Centaurea jacea*	*Succisa pratensis*
Plant size (z_1)	1.00	0.81	1.68	1.44	1.34
Additional threshold size for flowering (z_2)	0.00	0.00	0.00	0.48	0.00
Seed production per unit plant size (z_3)	1.00	1.00	1.93	2.02	2.48
Direct effect on plant survival and clonal propagation (v_1)	1.00	0.53	0.15	1.00	1.00
Direct effect on seedling establishment (v_2)	1.00	0.00	1.00	0.00	0.31

Figure 1.



stage at time *t*

stage at time *t+1*	sdl	veg	flow	side.veg	side.flow	
sdl	-	-	$w_9 w_{10}$	-	$w_9 w_{10}$	F = Sexual repr.
veg	w_1	$w_2(1-w_6)$	$w_3(1-w_7)$	$w_2(1-w_6)$	$w_3(1-w_7)$	S = Survival
flow	-	$w_2 w_6$	$w_3 w_7$	$w_2 w_6$	$w_3 w_7$	
side.veg	-	$w_4(1-w_8)$	$w_5(1-w_8)$	$w_4(1-w_8)$	$w_5(1-w_8)$	C = Clonal prop.
side.flow	-	$w_4 w_8$	$w_5 w_8$	$w_4 w_8$	$w_5 w_8$	

Figure 1. (top) Hierarchical population model of the contributions of nutrient enrichment (environmental factor) to consecutively plant traits, vital rates, matrix elements and population growth (see Table 1 for definitions of all model parameters). Eutrophication affects plant traits (z_1, z_2, z_3) but also directly (v_1, v_2) affects the survival and establishment rates independent of changes in plant traits, for instance through changes in the surrounding vegetation. In this case we made the simplifying assumption that the investigated plant traits do not co-vary. (bottom) 5×5 1-year transition matrix with 10 vital rates (w_k). The top row contains sexual reproduction, the second and third rows contain survival elements, and the bottom two rows contain clonal propagation

Figure 2.

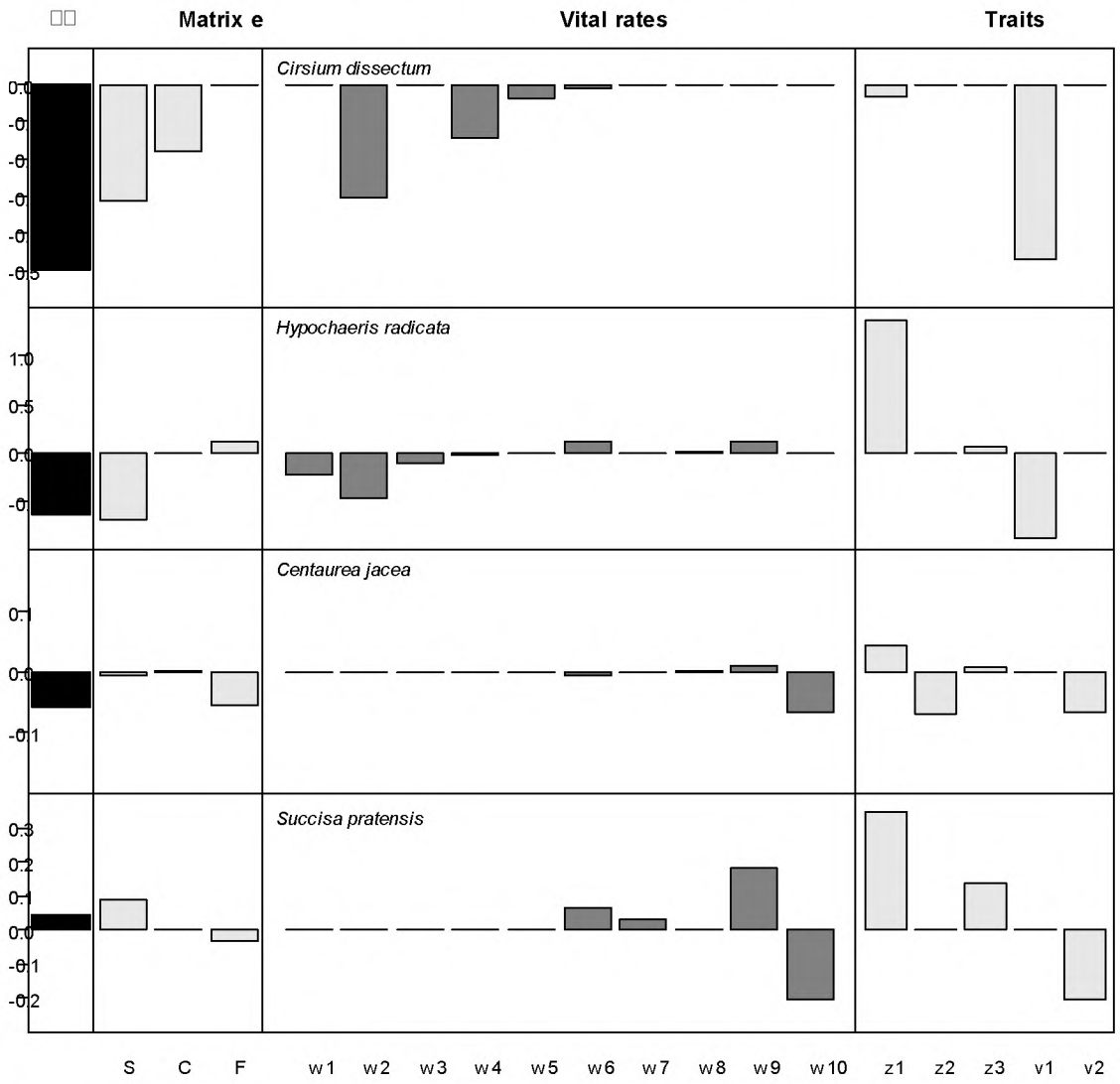
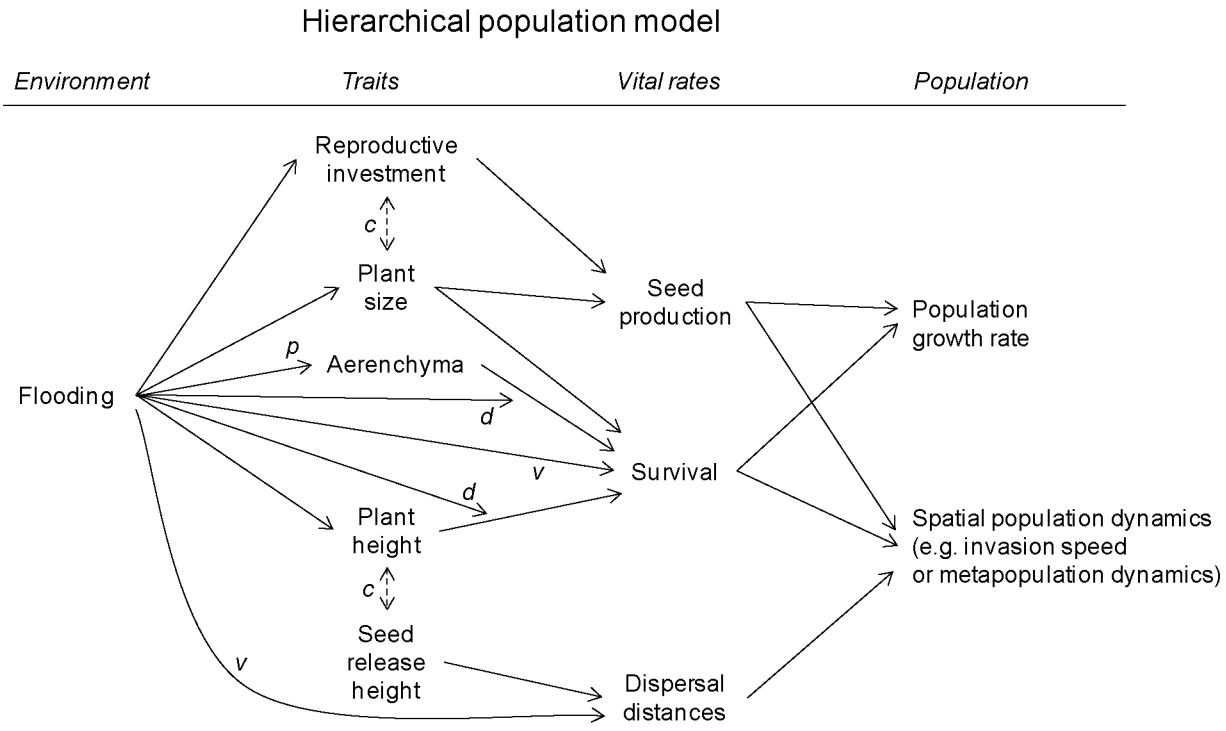


Figure 2. For each of four grassland herb species (rows): Decomposition (LTRE) of the differences in projected population growth ($\Delta\lambda$, between the nutrient enriched scenario and the default field scenario) into contributions of differences in model parameters at three different levels: groups of matrix elements (S, C, F), vital rates (w_k), and plant traits (z_k) (see Table 1 for an explanation of the vital rates and plant traits). The matrix elements are grouped as in Fig. 1: F = sexual reproduction, S = survival, C = clonal propagation. At the plant trait level $\Delta\lambda$ is not only decomposed into contributions of the changes in the plants traits themselves (z_k), but also into contributions of the effects of the environment (v_h) on vital rates that do not involve plant traits. In each of the panels the LTRE contributions together approximate $\Delta\lambda$ given at the left.

570 **Figure 3**.



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573 **Figure 3**. An example of a hierarchical population model, in which environmental factors affect
574 a population through a cascade of changes in traits of individuals and changes in vital rates. As
575 illustrated by the arrows with letters, flooding may impact a population in three different ways:
576 by (*v*) directly affecting vital rates, by (*p*) affected plant traits, or (*d*) modifying relationships
577 between vital rates and plant traits. The level of phenotypic plasticity determines how a trait
578 responds (*p*) to environmental changes (i.e. a reaction norm). The contribution of traits to vital
579 rates can change (*d*) with the environment: e.g. in this example the importance of plant height for
580 survival depends on how much a plant is flooded. The environment can also affect vital rates
581 directly (*v*), in addition to effects through plant or seed traits; a flood can increase dispersal
582 distances compared to an unflooded condition. Traits may co-vary (*c*), for instance through
583 allometry or trade-offs.
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Scaling up phenotypic plasticity with hierarchical population models

Eelke Jongejans, Heidrun Huber and Hans de Kroon

Supplementary Material

In this appendix we provide additional information about our analyses in the second case study 'Eutrophication effects on the population dynamics of 4 grassland species'. In that case study we constructed a hierarchical population model (HPM) to investigate the effect of eutrophication on plant traits, vital rates, matrix elements and ultimately on projected population growth. The HPM for the 4 grassland species can be found in Figure 1 of the main text. We compared two scenarios: the control, field scenario that was based on demographic data from a single field site, and an eutrophication scenario that was based on the field scenario but in which plant traits and certain vital rates were changed proportionally to effect sizes found in a fertilization experiment and by comparing a range of field sites, as we will explain in this appendix.

To establish a default field scenario we use demographic data for all four species from the same permanent plots in a single, ca 1 ha grassland nature reserve: Konijnendijk (52°02' N, 6°26'E). Further details of the demographic observations can be found in Jongejans and de Kroon (2005) and Jongejans et al. (2008). Data on the fate of individuals were pooled over the four annual transitions from 1999 to 2003 to get time-insensitive estimations of the behavior of plants of these 4 species in this site. Because our aim was to explicitly investigate the role of plant size, we chose to reconstruct the previously published 6×6 matrix models. In this study we use a 5×5 matrix model in which the 5 stage classifications are not based on plant size, but entirely on survival, clonal propagation, flowering and sexual reproduction: seedlings (*sdl*) and new side rosettes (*side.veg*) can become adults after one year or die, and adults and side rosettes can either flower (*flow* and *side.flow*) or stay vegetative (*veg* and *side.veg*) the next year (see also Table 1 and Fig. 1 in the main text).

In total we defined 10 different vital rates (see Table 1): survival rates (w_1, w_2, w_3), clonal propagation rates (w_4, w_5) and flowering probabilities of surviving plants (w_6, w_7, w_8) of the various stage classes, as well as the seed production rate of flowering plants (w_9) and the seedling establishment rate (w_{10}). The next step was to quantify these 10 vital rates with the demographic field data using linear regression models with plant size (z_1) as explanatory variable (Table 1A). Plant size was quantified non-destructively by the product of the number of leaves and the maximum leaf length. For seed production and clonal propagation we used linear regressions of the form:

$$w_k^c = a_k^c z_1 + b_k^c \qquad (1)$$

in which a_k and b_k are regression parameters and the C-index indicates the control, field scenario. For the adult survival and flowering probabilities we performed generalized linear regression with a logit-link:

$$w_k^c = \frac{\exp\left(a_k^c z_1 + b_k^c\right)}{1+\exp\left(a_k^c z_1 + b_k^c\right)} \qquad (2)$$

Seedling establishment (w_{10}) and seedling survival (w_1) were derived from a seed addition experiment (Soons et al. 2005; Jongejans et al. 2006b) and were not constructed as functions of plant size but as constants. In *C. dissectum* w_5, w_8 and w_9 were also constant because this species only forms one flower head per flowering rosette and because the data set on clonal propagation by flowering rosettes and the consecutive flowering probability of the new clonal offspring was too small to allow for regression analyses (Jongejans et al. 2008).

Two other traits beside plant size were defined as well. These two traits shape the relationship between the amount of sexual reproduction and plant size. The threshold size for flowering (z_2) was not estimated in the logistic regression analysis of the demographic data, but since the flowering threshold was sometimes affected by experimental nutrient enrichment, we defined z_2 as a reduction in plant size in the flowering probability function. $z_2 = 0$ was the default value for the control, field scenario. The last plant trait, seed production per unit plant size (z_3), determined the slope of the sexual reproduction – plant size relationship. This trait (z_3) is therefore by definition equal to the regression parameter a_9 in the linear regression model for seed production.

Table A1. Vital rate (w_k) regression models and eutrophication factors (f).
Linear regression models (with our without logit-link) of vital rates are presented as functions of rosette size (z_1) and regression parameters a and b. The number of plants in each analysis (n), the mean rosette size of the used subsample, the significance of the parameters (p_a and p_b) and the explained variance (R^2) are also given, as well as the mean vital rate value calculated for the control, field scenario. The location of the f-factors in the table indicate where the five different eutrophication factors are inserted in the vital rate calculations.

w_k	Regression	Rosette size (z_1)		Regression parameters					$\bar{w}_k$
		n	mean	*a*	*b*	p_a	p_b	R^2	
				Cirsium dissectum					
w_1	constant								0.450 $\cdot f_4$
w_2	logit	825	32.8 $\cdot f_1$	0.016	-0.012	0.003	0.950	0.015	0.625 $\cdot f_4$
w_3	constant								0.000
w_4	linear	22	28.9	-0.003	0.411	0.612	0.033	0.012	0.328 $\cdot f_4$
w_5	constant								2.481 $\cdot f_4$
w_6	logit	514	33.9 $\cdot f_1$	0.045	-4.369	0.000	0.000	0.081	0.056
w_7	constant								0.000
w_8	constant								0.006
w_9	constant								31.88
w_{10}	constant								0.001 $\cdot f_5$
				Hypochaeris radicata					
w_1	constant								0.539 $\cdot f_4$
w_2	logit	465	31.1 $\cdot f_1$	0.015	0.531	0.012	0.009	0.022	0.733 $\cdot f_4$
w_3	logit	89	46.6 $\cdot f_1$	0.058	-1.599	0.001	0.032	0.218	0.753 $\cdot f_4$
w_4	linear	465	31.1	0.001	0.005	0.086	0.774	0.006	0.028 $\cdot f_4$
w_5	linear	89	46.6 $\cdot f_1$	0.002	-0.072	0.023	0.147	0.058	0.034 $\cdot f_4$
w_6	logit	339	32.5 $\cdot f_1$	0.053	-3.412	0.000	0.000	0.229	0.158
w_7	logit	63	51.2	0.020	-1.789	0.180	0.030	0.040	0.313
w_8	logit	17	48.8 $\cdot f_1$	0.124	-8.730	0.035	0.025	0.608	0.065
w_9	linear	89	46.6 $\cdot f_1$	1.490 $\cdot f_3$	63.67	0.003	0.010	0.100	133.1
w_{10}	constant								0.019 $\cdot f_5$
				Centaurea jacea					
w_1	constant								0.897 $\cdot f_4$
w_2	logit	350	41.3	0.009	0.641	0.147	0.015	0.009	0.730 $\cdot f_4$
w_3	constant								0.000
w_4	linear	350	41.3	0.001	0.158	0.575	0.005	0.001	0.186 $\cdot f_4$
w_5	linear	136	31.1	0.003	0.864	0.072	0.000	0.024	0.956 $\cdot f_4$
w_6	logit	255	42.3 $\cdot f_1 \cdot (1-f_2)$	0.029	-2.064	0.000	0.000	0.112	0.306
w_7	constant								0.000
w_8	logit	195	39.3 $\cdot f_1 \cdot (1-f_2)$	-0.015	-0.087	0.002	0.689	0.081	0.334
w_9	linear	136	31.1 $\cdot f_1$	0.186 $\cdot f_3$	22.88	0.000	0.000	0.195	28.669
w_{10}	constant								0.008 $\cdot f_5$
				Succisa pratensis					
w_1	constant								0.858 $\cdot f_4$
w_2	logit	750	35.2	0.008	2.267	0.345	0.000	0.003	0.929 $\cdot f_4$
w_3	logit	208	44.9	-0.029	4.084	0.105	0.000	0.033	0.942 $\cdot f_4$
w_4	linear	750	35.2	0.000	0.082	0.697	0.001	0.000	0.073 $\cdot f_4$
w_5	linear	208	44.9	0.000	0.085	0.921	0.197	0.000	0.091 $\cdot f_4$
w_6	logit	696	35.4 $\cdot f_1$	0.047	-2.961	0.000	0.000	0.156	0.216
w_7	logit	195	44.5 $\cdot f_1$	0.042	-2.073	0.000	0.000	0.107	0.448
w_8	logit	74	37.1 $\cdot f_1$	0.041	-4.423	0.048	0.000	0.128	0.051
w_9	linear	208	44.9 $\cdot f_1$	1.099 $\cdot f_3$	21.73	0.015	0.310	0.028	71.08
w_{10}	constant								0.023 $\cdot f_5$

Five different effects of increased nutrient availability on the population dynamics were considered: three via the above-mentioned plant traits and two effects directly through vital rates. Most of these effects were determined in a previous study in which the effect of nutrient enrichment on the survival, size and allocation patterns of the four studied grassland species were investigated while they grew in the middle of dominating grasses (*Molinia caerulea*) in an experimental garden (Hartemink et al. 2004; Jongejans et al. 2006a). In order to translate the results of this experiment to the field situation, we calculated the eutrophication impacts (f) in plant traits and vital rates from the experiment and implemented these in the eutrophication scenario (which was based on the field scenario) as follows:

- eutrophication impact f_1 on plant size (z_1). In the experiment vegetative plant size was estimated by the total vegetative biomass (roots, leaves and stems). The effect on plant size was determined for the plants that survived until the end of the three-year experiment and estimated by the relative increase in vegetative biomass in the nutrient enrichment treatment compared to the control, no fertilization treatment. As can be seen in Table A1, regression models with plant size (as we measured it) did not significantly explain variation in all vital rates. Therefore we conservatively applied f_1 only in vital rate functions in which plant size was a significant parameter (i.e. $p_a < 0.05$). The functions of those vital rates were modified by multiplying z_1 by the eutrophication factor f_1; for instance in the function of the flowering probability of vegetative *Succisa pratensis* rosettes (the E-index indicates the eutrophication scenario):

$$w_6^E = \frac{\exp\left(a_6^C(z_1 f_1) + b_6^C\right)}{1+\exp\left(a_6^C(z_1 f_1) + b_6^C\right)} \quad (3)$$

- eutrophication impact f_2 on the threshold size for flowering (z_2). The threshold size for flowering was determined by the intercept of linear regressions of sexual reproductive biomass on vegetative plant biomass, in the fertilized and untreated groups separately. The value of f_2 was then estimated by the increase in a relative measure of z_2: the ratio of the intercept in the regression and the mean vegetative biomass. Nutrient addition affected z_2 only in *Centaurea jacea*. The flowering probability rates w_6 and w_8 of that species were modified by setting z_2 to a fraction f_2 of z_1 (f_2 was zero in all other cases):

$$w_6^E = \frac{\exp\left(a_6^C(f_1(z_1 - z_2^E)) + b_6^C\right)}{1+\exp\left(a_6^C(f_1(z_1 - z_2^E)) + b_6^C\right)} = \frac{\exp\left(a_6^C(z_1 f_1(1-f_2)) + b_6^C\right)}{1+\exp\left(a_6^C(z_1 f_1(1-f_2)) + b_6^C\right)} \quad (4)$$

- eutrophication impact f_3 on seed production per unit plant size (z_3). The slope of the regression of sexual reproductive biomass on vegetative biomass changed significantly in three species (but not in *Cirsium dissectum*). In these three species the function of seed production (w_9) was modified as follows: (5)

$$w_9^E = (a_9^C f_3)(z_1 f_1) + b_9^C = (z_3 f_3)(z_1 f_1) + b_9^C \quad (6)$$

- eutrophication impact f_4 (= v_1 in Tables 1 and 2 and Figs. 1 and 2) on survival and clonal propagation (w_1, w_2, w_3, w_4 and w_5). The direct effect of nutrient enrichment on the survival of one-year old seedlings (w_1) was not investigated separately. Therefore we assumed that the seedling survival rate was affected in the same way as the adult survival rate. The ratio of the number of surviving plants after three years in the nutrient enrichment treatment compared to the number of surviving plants in the unfertilized group was used as an estimate of the effect of extra nutrients on adult survival and clonal

propagation. We assumed that beside rosette survival, clonal propagation by these rosettes was affected by the same factor because clonal propagation involves annual survival too. The impact of this direct effect of eutrophication on vital rates was modeled by multiplying the average value of a vital rate by f_4; for instance in the case of the survival of *Hypochaeris radicata* seedlings:

$$w_1^E = w_1^C f_4 \quad (7)$$

- eutrophication impact f_5 (= v_2 in Tables 1 and 2 and Figs. 1 and 2) on seedling establishment (w_{10}). The effect of high productivity on seedling establishment was investigated with the above-mentioned seed addition experiment. Here we use the ratio of the establishment rate in high productive sites (habitat class 2; Soons et al. 2005) compared to the establishment rate in low productive sites (habitat class 1) as an estimate of the effect of nutrient enrichment. The eutrophication factor f_5 was included in the calculation of w_{10}^E in a similar way as was done for f_4 in equation 7.

The *f*-values can be found in Table 2 in the main text. Finally, the field scenario vital rates (w_k^C) and eutrophication scenario vitral rates (w_k^E) were used to construct transition matrix models as is shown in the bottom of Fig. 1.

LTRE analyses of the impact of eutrophication on population dynamics

Life table response experiments (a variance decomposition technique) were used to investigate which differences in model parameters had the highest contributions to the difference ($\Delta\lambda$) between the projected population growth rate of the eutrophication scenario (λ^E) and that of the default field scenario (λ^C). First we decomposed $\Delta\lambda$ at the level of the matrix elements (a_{ij}), then at the level of the underlying vital rates (w_k), and finally at the level of the involved plant traits (z_r). The last level also included the contributions of changes in direct environmental effects (v_h) on vital rates, which effects circumvent plant traits. The LTRE models that approximate $\Delta\lambda$ were:

$$\Delta\lambda = \lambda^E - \lambda^C \quad (8)$$

$$\cong \sum_{ij}\left(a_{ij}^E - a_{ij}^C\right)\frac{\partial\lambda}{\partial a_{ij}}\bigg|_{\frac{1}{2}\left(A^E + A^C\right)} \quad (9)$$

$$\cong \sum_{k}\left(w_k^E - w_k^C\right)\frac{\partial\lambda}{\partial a_{ij}}\frac{\partial a_{ij}}{\partial w_k}\bigg|_{\frac{1}{2}\left(A^E + A^C\right)} \quad (10)$$

$$\cong \sum_{r}\left(z_r^E - z_r^C\right)\frac{\partial\lambda}{\partial a_{ij}}\frac{\partial a_{ij}}{\partial w_k}\frac{\partial w_k}{\partial z_r}\bigg|_{\frac{1}{2}\left(A^E + A^C\right)} + \sum_{h}\left(v_h^E - v_h^C\right)\frac{\partial\lambda}{\partial a_{ij}}\frac{\partial a_{ij}}{\partial w_k}\frac{\partial w_k}{\partial v_h}\bigg|_{\frac{1}{2}\left(A^E + A^C\right)} \quad (11)$$

The LTRE models should approximate $\Delta\lambda$ at each of these levels because the only source of deviations between the λ's of the field and eutrophication scenarios was the difference in the environmental factor eutrophication (i.e. at the lowest level of the HPM). The LTRE models fitted well in general (i.e. the bars in each of the panels in Fig. 2 sum up to a number close to $\Delta\lambda$ for the respective species), except for the plant trait level in *H. radicata* and *S. pratensis*, where plant size has a disproportionally large contribution, potentially because of non-linearities in the LTRE analyses.

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