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Plasticity in response to phosphorus and light availability in four forest herbs

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Abstract

The differential ability of forest herbs to colonize secondary forests on former agricultural land is generally attributed to different rates of dispersal. After propagule arrival, however, establishing individuals still have to cope with abiotic soil legacies from former agricultural land use. We focussed on the plastic responses of forest herbs to increased phosphorus availability, as phosphorus is commonly found to be persistently bioavailable in post-agricultural forest soils. In a pot experiment under field conditions, we applied three P levels to four forest herbs with contrasting colonization capacities: *Anemone nemorosa*, *Primula elatior*, *Circaea lutetiana* and *Geum urbanum*. To test interactions with light availability, half of the replicas were covered with shade cloths. After two growing seasons, we measured aboveground P uptake, vegetative and regenerative performance. We hypothesized that fast-colonizing species respond the most opportunistically to increased P availability and that a low light availability can mask the effects of P on performance. All species showed a significant increase in P uptake in the aboveground biomass. The P addition had a positive effect on the vegetative performance of two of the species, which was, however, unrelated to their colonization capacities. The regenerative performance was affected by light availability (not by P addition) and was related to the species' phenology. Forest herbs can obviously benefit from the increased P availabilities in post-agricultural forests, but not all species respond in the same way. Such differential patterns of plasticity may be important in community dynamics as they affect the interactions among species.

Key-words: secondary succession, post-agricultural forest, pot experiment, plant performance, bioavailability of P

Introduction

Plants can cope with spatio-temporal environmental variation by adjusting their growth and development. The ability of a given genotype to render different phenotypes subject to the environmental conditions is known as phenotypic plasticity (Bradshaw 1965; Schlichting 1986; Sultan 2000). Because a high plasticity enhances the match between phenotype and environment, an ideal species would exhibit 'infinite' plasticity, i.e., expressing optimal trait values in every environment. Yet, there are ecological limitations to phenotypic plasticity (DeWitt et al. 1998; Valladares et al. 2007), and individuals of different taxa and functional plant types may differ remarkably in the extent of their response to environmental variation (e.g., Grime et al. 1997; Valladares et al. 2006). For traits that directly relate to growth and reproduction, i.e., components of fitness, differences in plasticity between species may contribute to differences in their realized distributions. In particular, the breadth of a species' distribution reflects its ability to both maintain high performance under limited resource availability and opportunistically maximize performance under favourable conditions (cf. Sultan 2001; Griffith and Sultan 2005). The ecological consequence is that the capacity of a species to maintain populations across a range of environments contributes to its ability to thrive in alternative environments or colonize and persist in novel sites (e.g., Donohue et al. 2001).

Forests on former agricultural land are a particular example of communities in which species have to cope with alternative, novel environmental conditions. Massive clearance of forests for agriculture followed by land abandonment and re-establishment of forests over the past centuries has created complex landscapes dominated by post-agricultural forest in much of Europe and north-eastern North America (reviews: Flinn and Vellend 2005; Gilliam 2007; Hermy and Verheyen 2007). There is ample evidence of persistent (a)biotic differences between post-agricultural stands and forest stands for which there is no historical record of alternative land use ('ancient forest'). The abiotic legacies of former land use are expressed in soil nutrient availability (e.g., Koerner et al. 1997; Verheyen et al. 1999; Falkengren-Grerup et al. 2006) and spatial heterogeneity of soil properties (Fraterrigo et al. 2005; Flinn and Marks 2007). Among other nutrients, phosphorus (P) has specifically drawn the attention. A P surplus from former fertilization was found several decades (Koerner et al. 1997; Verheyen et al. 1999; De Keersmaecker et al. 2004) or even millennia (Dambrine et al. 2007) after post-agricultural forest establishment. In addition, Richter et al. (2006) found an extensive redistribution of P among the different pools in the soil, but remarkable little depletions in most of the bioavailable P fractions during 28 year of forest development on former fields. Therefore, the persistent, bioavailable P pool may generate new challenges for forest herbs that colonize post-agricultural sites. The importance of a persistent P surplus should also be considered in the face of the chronic deposition of nitrogen (N) and the impact of N on temperate forest vegetations (Gilliam 2007; Gress et al. 2007). Gress et al. (2007), for instance, showed that an increasing N availability increased the demand for P and predicted that that P limitation will become an important control over the forest vegetation dynamics. A large pool of bioavailable P might cancel out this limitation.

Forest herbs greatly differ in their capacity to colonize post-agricultural forests (Verheyen et al. 2003). Some colonize and persist soon after the establishment of a woody canopy, but others fail to do so for centuries. Although several lines of evidence demonstrate that different rates of dispersal shape the initial colonization patterns (Honnay et al. 2002; Verheyen et al. 2003; Flinn and Vellend 2005), the contrasting colonization capacity among species might be accentuated if fast dispersers also have

the strongest tendency to maximize performance in novel environments. A number of field studies found effects of previous agricultural land use on several components of performance in forest herbs (e.g., Endels et al. 2004; Verheyen and Hermy 2004; Fratterigo et al. 2006; Baeten et al. 2009). Despite the difference in P availability between the ancient and post-agricultural sites, the cited studies failed to prove that P is the actual environmental factor that induced the phenotypic responses because it was confounded with several other environmental legacies from former land use (e.g., pH, nitrogen and light availability). Some experimental studies provide more insight into this matter. Pigott & Taylor (1964) and Rorison (1968) found positive responses to P addition, in term of biomass, for seedlings of some forest herbs. More recent experimental work by Hipps et al. (2005) showed similar patterns: in a glasshouse bioassay with four forest herbs growing on peat-based compost, they found species-specific effects of P addition on the P uptake and biomass of the forest herbs. Following the recommendations to perform plasticity studies in ecologically relevant, natural settings and multifactor environments (Sultan 2000; Miner et al. 2005; Valladares et al. 2007), this study aims to extend the previous experimental work by introducing some real-world complexities. We performed a pot experiment with forest soil instead of compost, the individuals were placed under field conditions and the experiment was continued for two growing seasons. Apart from P, we also manipulated light availability. Light is not only a pivotal resource in forest ecosystems, it may also constrain growth and mask the effect of P addition on growth and performance. A number of studies, for instance, found a positive effect of nutrient addition on the growth of forest herbs, but only if light availability was not limiting (Eickmeier and Schussler 1993; Meekins and McCarthy 2000; Anderson 2003; Elemans 2004). We measured P uptake and biomass (cf. Rorison 1968; Hipps et al. 2005), and also recorded a suite of plant traits related to growth and reproduction. Finally, the four forest herbs used in our study strongly differ in their capacity to colonize post-agricultural forest (Verheyen et al. 2003, Table 1), which enabled us to test whether fast colonizers actually respond stronger to P availability than slow colonizers. We hypothesized that (1) the four forest herbs show plastic responses to P and light availability, (2) light and P availability interactively affect the performance of individuals and (3) the fast-colonizing species are more plastic than the slow-colonizing species.

Methods

Study species and experiment initiation

We used four common herbaceous forest species with a similar distribution in mesotrophic, deciduous forests in Western Europe. The four study species were specifically chosen to represent different life forms and different leaf and flowering phenologies (Table 1). Furthermore, the species differ in their affinity to historically continuous 'ancient' forest. *Anemone nemorosa* L. is the most dependent on forest continuity whereas *Geum urbanum* L. occurs frequently in post-agricultural forest as well. *Primula elatior* (L.) Hill and *Circaea lutetiana* L. have intermediate capacities to colonize post-agricultural forests (Verheyen et al. 2003). Individuals or ramets (for geophytes) of the four study species were grown from seeds (*P. elatior*, *G. urbanum*) or rhizomes (*A. nemorosa*) and hibernacles (*C. lutetiana*, Verburg and Grava 1998) in small pots (0.6 litre) filled with compost soil during early spring 2007. No extra nutrients were added. We used four rhizome fragments in each pot for both *C. lutetiana* and *A. nemorosa*. Theoretically, phenotypic plasticity should be studied using genetically identical individuals that are exposed to the experimental treatments. This is,

however, difficult to achieve in ecological studies, and plasticity is often measured in a more general sense (Valladares et al. 2006). We aimed to have similar levels of genetic variability across the species by collecting the seeds or ramets of each species in large populations of ancient forest sites in the same locality (Vlaams Brabant region). Furthermore, because the grown seedlings were completely randomized over the treatments, the uncontrolled intraspecific genetic differences will be captured by the unexplained variance of our statistical models.

Table 1: Life form, phenology and colonization capacity of the four forest herbs used in the study

Species	Family	Life form	Flowering period	Leaf phenology	Colonization capacity*
<i>Anemone nemorosa</i>	Ranunculaceae	Geophyte (rhizome)	Spring	Vernal	Low
<i>Primula elatior</i>	Primulaceae	Hemicryptophyte (rosette)	Spring	Vernal-aestival	Moderate
<i>Circaea lutetiana</i>	Onagraceae	Geophyte (hibernacle)	Summer	Aestival	Moderate
<i>Geum urbanum</i>	Rosaceae	Hemicryptophyte (rosette)	Summer	Winter green	High

* Based on data from Verheyen et al. (2003).

In the first week of May 2007, the four species were transplanted into 3 litre pots (N = 36 per species) that were filled with soil with a sandy silt texture (mean dry weight: 1063 g soil/pot \pm 17 SE, N = 10). The soil was collected in the forest 'Muizenbos' (51.197 N, 4.566 E), which is a mesotrophic forest characterized by a complex mosaic of stands with well-documented, contrasting land-use histories (e.g., Verheyen and Hermy 2001). To assess whether soil factors other than P availability may also induce variation in plant performance, soil from two adjacent forest stands with a contrasting land-use history (ancient versus post-agricultural) but similar, low P concentrations was used to fill half of the pots each (N = 18 per species). The selection of the two stands was based on detailed analyses of the chemical soil conditions, notably P (De Keersmaecker et al. 2004), and land-use history (Verheyen and Hermy 2001). The first stand has known continuous forest cover for at least 150 years whereas the second stand had been used as extensive agricultural land until it was forested in the 1950s. The dominant species in the tree layer were *Populus x canadensis* in the ancient forest stand and *Fraxinus excelsior* L. in the post-agricultural stand; *Corylus avellana* L. dominated the coppice layer. The soil for the pot experiment was collected by digging up the upper 5 cm of mineral soil in ten patches of 1–2 m² in both stands. For each of the stands, the sampled soil was profoundly mixed, and the two soil types were used to fill the 18 pots for each species. After transplantation, the species were placed under equal forest conditions for the rest of the growing season to recover from transplantation stress. To characterize initial differences among individuals, we measured the number of leaves and the length and width of the three largest leaves in each pot just after transplantation (May 2007 for the spring-flowering species, August 2007 for the summer-flowering species).

To characterize the chemical soil conditions, we analysed four samples of the soil of both stands. The soil of the ancient forest stand had an average pH-KCl of 5.2 (\pm 0.1 SE); ammonium-acetate EDTA extractable (mg/100 g dry soil) K⁺ 13.1 (\pm 0.6), Mg²⁺ 11.9 (\pm 0.8), Ca²⁺ 268.6 (\pm 3.1) and P 0.5 (\pm 0.07)

and Kjeldahl N 235.9 (\pm 12.4) mg/100 g dry soil. The soil collected in the post-agricultural forest stand was characterized by a pH 4.8 (\pm 0.01), K⁺ 6.4 (\pm 0.06), Mg²⁺ 6.9 (\pm 0.2), Ca²⁺ 230.4 (\pm 8.4), P 0.6 (\pm 0.04) and N 241.1 (\pm 3.7) mg/100 g dry soil.

Experimental design and treatments

In January 2008, all pots were placed under field conditions in a homogeneous *Fraxinus excelsior* (tree layer) and *Tilia cordata* Mill. (shrub layer) dominated 1.38 ha forest stand of the ancient forest 'Aelmoeseneiebos' (50.975 N, 3.795 E). For each species, we placed three pots with ancient forest soil and three pots with post-agricultural forest soil into each of six fenced blocks (3 pots x 2 soil types x 6 blocks \sim N = 36 per species). To simulate two levels of light availability, half of the blocks were covered with one layer of shade cloth placed at 110 cm height (70 % light reduction, polyethylene) when the canopy started leafing out (third week of April). The shade cloth was removed when the leaves started falling off (third week of October). Within each block, we applied three different P levels (fully crossed with the two soil types): control, 50 mg P/pot and 210 mg P/pot. These levels were in the same order of magnitude as in the previous work by Pigott and Taylor (1964) and Hipps et al. (2005). Adjusted solutions of NaH₂PO₄ were used as P source. To account for biogeochemical P fixation in the soil, we applied 50 mL of the NaH₂PO₄ solutions at four occasions during the growing season (ten day intervals). Applications started at emergence (geophytes) or new leaf development (hemicryptophytes). The shade and P treatments were applied during the growing season of 2008 and 2009.

Measurement of performance and P uptake

We determined plant performance by measuring a suite of species-specific traits related to growth and reproduction (cf. Endels et al. 2004; Verheyen and Hermy 2004; Baeten et al. 2009). The vegetative traits (except for *A. nemorosa*) were: total number of leaves, leaf length and total aboveground dry biomass. For ramets of *A. nemorosa*, we measured the leaf length and width, height of the leaf above the surface and biomass. The regenerative traits were: inflorescence height, number of inflorescences (not for *A. nemorosa*) and the number of flowers (*G. urbanum*, *P. elatior*) or the proportion of flowering ramets (*A. nemorosa*, *C. lutetiana*). The traits were measured on maximum three leaves and inflorescences per individual; for the geophytes, we always used the three tallest ramets. All measurements were done just following peak flowering of each species: 28 Apr 2009 (*A. nemorosa*, *P. elatior*), 16 Jun 2009 (*G. urbanum*) and 7 Jul 2009 (*C. lutetiana*). We also determined P in the aboveground biomass to assess whether the P added to the pots was actually bioavailable and utilized by the plants and to relate the results with data from literature. Therefore, the total aboveground biomass was harvested directly after we measured the trait values, dried for 72 hours at 70°C and weighted (\pm 0.1 mg). The total P concentrations (mg P/100 g dry biomass) were measured colorimetrically by the molybdate method (Scheel 1936) following a di-acid digestion (HNO₃ 65 %, HClO₄ 70 %, 5:1). The total aboveground P content (mg P) of individuals or ramets was calculated based on the dry biomass and the P concentration.

Data analysis

For each species, we calculated four different response variables that were evaluated against the experimental factors. First, we looked at P pools in the aboveground biomass: P concentration and total P content (concentration x total biomass) of individuals or ramets. Next, we performed a

principal component analysis (PCA) on the vegetative and regenerative traits separately and extracted the first axes as measures of vegetative and regenerative performance (cf. Baeten et al. 2009). When appropriate, the trait values were log-transformed to increase the proportion of variance extracted. For each species, the first axes extracted > 61 % of either the variation in vegetative or regenerative traits, and each trait value showed a positive correlation with its associated PCA axis, i.e. there were no trade-offs between traits. The mean Pearson correlation coefficient between a trait and the first PCA axis was 0.84 (± 0.11 SD, all significant at $P < 0.0001$) for the vegetative traits and 0.90 (± 0.06 SD, all significant at $P < 0.0001$) for the regenerative traits. To account for initial differences in performance among individuals, we calculated the plant performance just following transplantation, i.e., the first axis of a PCA on the 2007 measurements (number of leaves, length and width of the longest leaf). The initial plant performance variable (INITIAL PERF) was used as a covariate in the analysis of the two response variables vegetative and regenerative performance.

The experimental design was hierarchical with the shade treatment at the block level and the P treatment at the pot level. Therefore, we applied multilevel models using the lme function in the nlme library of R version 2.9.1 (R Development Core Team 2009) with 'block' included as random effect term. We compared competing, nested models with the likelihood ratio test. The philosophy of this method is to compare the degree of belief in the competing models, rather than selecting a single model based on p-values (Hilborn and Mangel 1997). First, we constructed a null model with the intercepts varying randomly by block; the model parameters were maximum likelihood estimates (Model 0). The total variation in the response variable was divided among the pot level and the block level ('intra-class correlation'). Next, we added the factor SHADE, i.e., cover by shade cloth or not, to Model 0 and performed a likelihood ratio test to evaluate whether the factor significantly improved the null model. The significance of the factor P addition was tested in the same way. Because we were mainly interested in the experimental factors SHADE and P addition, we will always report the full factorial model with both these factors and their interaction in the results (Model 1). Finally, we added the covariate INITIAL PERF (initial plant performance) or the factor SOIL (soil originating from the ancient or post-agricultural stand) to Model 1 to test whether they could additionally account for variation in the response variable. The contribution of the variable INITIAL PERF or SOIL will only be reported if it, based on the likelihood ratio test, significantly improved Model 1. The covariate INITIAL PERF was not used in the models explaining P concentration and content. Prior to all multilevel analyses, Box-Cox transformations were applied to maximize the normality of the data.

Results

P concentration and total P content

Phosphorus addition had a strong effect on the P concentration and total P content in the aboveground tissue of the four forest herbs (Fig. 1, Table 2). Variability in P concentration and content was mainly related to the pot level; only for *G. urbanum*, P content also varied considerably (48 % of variation) among blocks. Imposing additional shade had a significant effect on P concentrations in the two summer-flowering herbs: a model that included the factor SHADE significantly improved the null model, based on the likelihood ratio test (Table 2a). In the blocks covered with a shade cloth, P concentrations tended to be higher compared to blocks without shade cloth. The P treatment was the most important factor explaining P concentrations in aboveground

plant tissue of all species. The effect was most pronounced for *C. lutetiana*. The mean P concentrations of the species ranged from 260 mg P/100 g dry biomass in the control to 695 mg P/100 g dry biomass in the highest P addition treatment, i.e., a 2.7-fold increase. The full factorial models explained > 63 % of the variation in P concentration for all species.

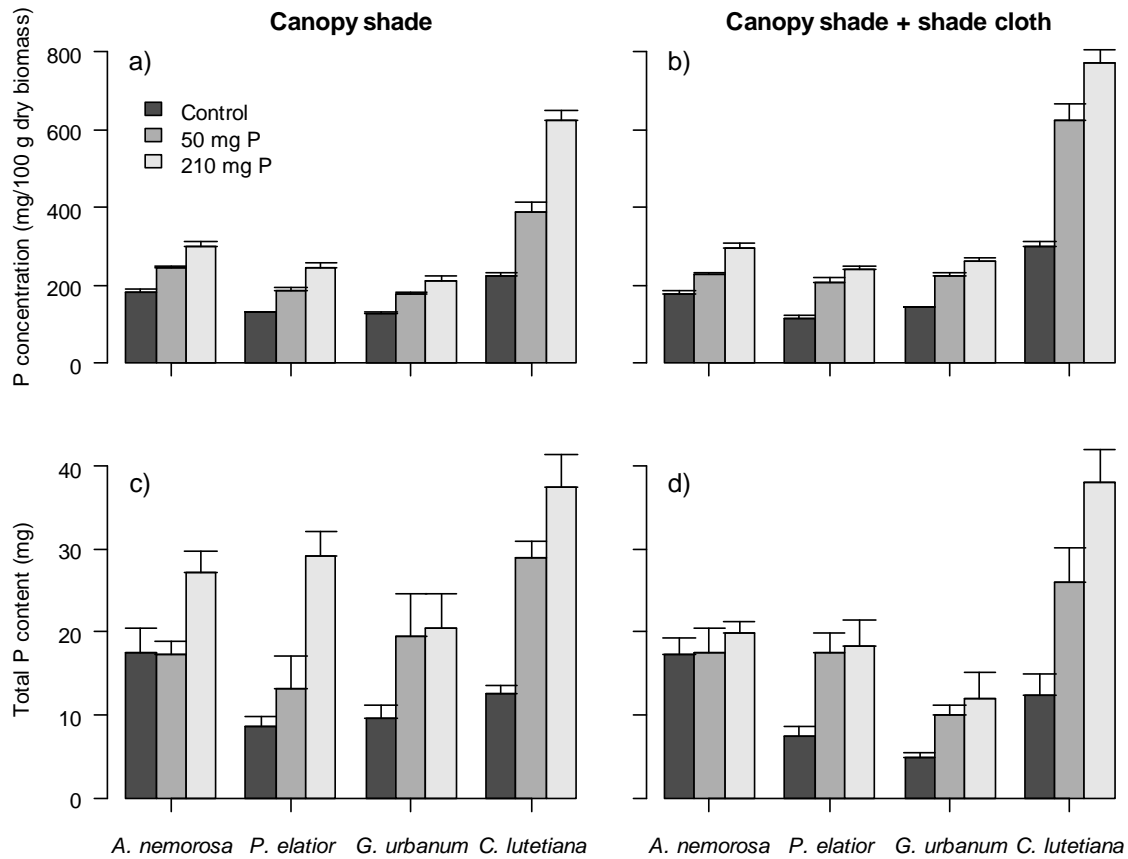


Fig. 1 Aboveground P concentrations (a, b) and total P content (c, d) of four forest herbs (mean \pm SE) in response to P addition and SHADE treatments (N = 36 per species). The total P content of *A. nemorosa* and *C. lutetiana* was rescaled to the content in 100 and 25 ramets, respectively. Results of the multilevel models are reported in Table 2

The total P content in the aboveground plant tissue showed similar responses (Table 2b). The P addition treatment was the main determinant for the variation in P content between individuals or ramets. The effect of SHADE was no longer significant for *C. lutetiana*, but tended to be still important for *G. urbanum*, which showed a higher total P content in blocks with just canopy shade (48 % variance at the block level of which 17 % was explained by the factor SHADE). Tissue P concentration below the shade cloths was higher, but total biomass was lower (cf. vegetative performance) compared to blocks without shade cloth, which partly levelled out the effect of SHADE on total P content. For the two geophytes *A. nemorosa* and *C. lutetiana*, introducing the factor SOIL further improved the model. The mean total P content of the two species was higher in the soil originating from the post-agricultural stand because of a higher biomass (cf. vegetative performance), not because of higher P concentrations.

Table 2: Effects of treatments (SHADE, P addition and SOIL) on the (a) aboveground P concentration and (b) total P content of four forest herbs tested with multilevel models. See methods for details on the model building.

	Spring-flowering		Summer-flowering	
	<i>A. nemorosa</i>	<i>P. elatior</i>	<i>G. urbanum</i>	<i>C. lutetiana</i>
a) P concentration				
Variance (explained)				
block level	< 0.1 %	< 0.1 %	< 0.1 %	< 0.1 %
pot level	> 99 % (63 %)	> 99 % (65 %)	> 99 % (66 %)	> 99 % (73 %)
Likelihood ratio				
L.Ratio _{SHADE}	0.2	< 0.1	5.0*	5.3*
L.Ratio _P	69.1***	65.3***	58.9***	62.2***
Source of variation				
SHADE	1.4	< 0.1	30.1**	53.5**
P	93.2***	99.7***	93.4***	146.6***
SHADE x P	0.4	2.6	2.8	4.8*
SOIL				6.4*
b) Total P content				
Variance (explained)				
block level	< 0.1 %	< 0.1 %	48 % (17 %)	< 0.1%
pot level	> 99 % (32 %)	> 99 % (36 %)	52 % (27 %)	> 99 % (50 %)
Likelihood ratio				
L.Ratio _{SHADE}	1.4	0.7	2.4	< 0.1
L.Ratio _P	8.7*	22.1***	16.9***	42.1***
Source of variation				
SHADE	1.4	1.4	2.5	0.1
P	6.6**	16.8***	10.0***	40.0***
SHADE x P	2.4	4.1*	0.7	0.2
SOIL	10.1**			4.6*

Notes: Variance = the fraction of the variation in P concentration or content at the block and pot level with the % variance explained by the model within parentheses. Likelihood ratio = test statistic and significance to test for whether SHADE (L.Ratio_{SHADE}) or P (L.Ratio_P) improves the null model with random intercepts (grouping factor 'block'). Source of variation = F-values and significance for the different factors. The F-values for SHADE are tested with 4 denominator d.f., the other factors with 26 denominator d.f.; *: p < 0.05; **: p < 0.01; ***: p < 0.001

Vegetative and regenerative performance

The vegetative performance of *P. elatior* and *C. lutetiana* was significantly higher in pots with experimental P addition (significant F-value and L.Ratio_P); *A. nemorosa* and *G. urbanum* did not significantly react to the P treatment (Table 3a, Fig. 2). SHADE affected the vegetative performance of the two summer-flowering species: 55 % and 15 % of the variation was at the block level of which 38 % and 64 % could be explained by including the factor SHADE. The vegetative performance of the spring-flowering species was unrelated to SHADE. The regenerative performance was largely unrelated to the P addition treatment (Table 3b, Fig. 3), but SHADE did have an effect. The regenerative performance of the summer-flowering species was considerably lower in blocks covered by a shade cloth. The majority of performance variation of *G. urbanum* and *C. lutetiana* was at the block level (69 % and 72 %), and the factor SHADE accounted for 54 % and 90 % of this variation, respectively. Adding the factor SHADE significantly improved the null model (cf. L.Ratio_{SHADE}). Furthermore, the variation in vegetative performance was not independent from the regenerative performance. The two axes of trait variation showed a significant positive correlation for all four species ($R^2 \geq 0.47$; $P < 0.001$).

Primula elatior was the only species for which the vegetative and regenerative performance after two growing seasons was still significantly related to its performance in the first growing season (INITIAL PERF). The covariable INITIAL PERF explained 18 % and 21 % of the variation in vegetative and regenerative performance of the species in addition to a model with P and SHADE. For the other species, the initial performance differences were no longer found after two growing seasons. *Anemone nemorosa* showed a clearly different vegetative and regenerative performance in pots with soil from the different forest stands: its performance was, on average, higher in pots filled with soil from the post-agricultural stand. For *C. lutetiana*, including the factor SOIL marginally improved the models (L.Ratio $P < 0.08$), which also suggests a higher vegetative and regenerative performance in the pots with post-agricultural soil.

Table 3: Effects of treatments (SHADE, P addition and SOIL) and initial plant performance (INITIAL PERF) on the (a) vegetative performance (first PCA axis of vegetative traits) and (b) regenerative performance (first PCA axis of regenerative traits) of four forest herbs tested with multilevel models. See methods for details on the model building.

	Spring-flowering		Summer-flowering	
	<i>A. nemorosa</i>	<i>P. elatior</i>	<i>G. urbanum</i>	<i>C. lutetiana</i>
a) Vegetative performance				
Variance (explained)				
block level	12 % (< 0.1 %)	< 0.1 %	55 % (38 %)	15 % (64 %)
pot level	88 % (53 %)	> 99 % (46 %)	45 % (5 %)	85 % (26%)
Likelihood ratio				
L.Ratio _{SHADE}	1.1	1.3	2.5	3.6
L.Ratio _P	2.6	7.3*	0.4	8.9*
Source of variation				
SHADE	0.9	1.6	2.6	4.2
P	2.1	4.8*	0.2	4.3*
SHADE x P	1.6	1.4	0.5	< 0.1
INITIAL PERF		9.2**		
SOIL	20.0***			
b) Regenerative performance				
Variance (explained)				
block level	6 % (48 %)	< 0.1 %	69 % (54 %)	72 % (90 %)
pot level	94 % (34 %)	> 99 % (40 %)	31 % (16 %)	28 % (13 %)
Likelihood ratio				
L.Ratio _{SHADE}	2.8	2.7	4.3*	11.6***
L.Ratio _P	2.9	2.9	4.3	3.2
Source of variation				
SHADE	3.0	3.5	5.3	29.5**
P	1.7	1.9	2.0	1.5
SHADE x P	1.7	1.9	0.3	0.4
INITIAL PERF		7.8**		
SOIL	5.9*			

Notes: Variance = the fraction of the variation in performance at the block and pot level with the % variance explained by the model within parenthesis. Likelihood ratio = test statistic and significance to test for whether SHADE (L.Ratio_{SHADE}) or P (L.Ratio_P) improves the null model with random intercepts (grouping factor 'block'). Source of variation = F-values and significance for the different factors or covariate. The F-values for SHADE are tested with 4 denominator d.f., the other factors with 26 denominator d.f.; *: p < 0.05; **: p < 0.01; ***: p < 0.001

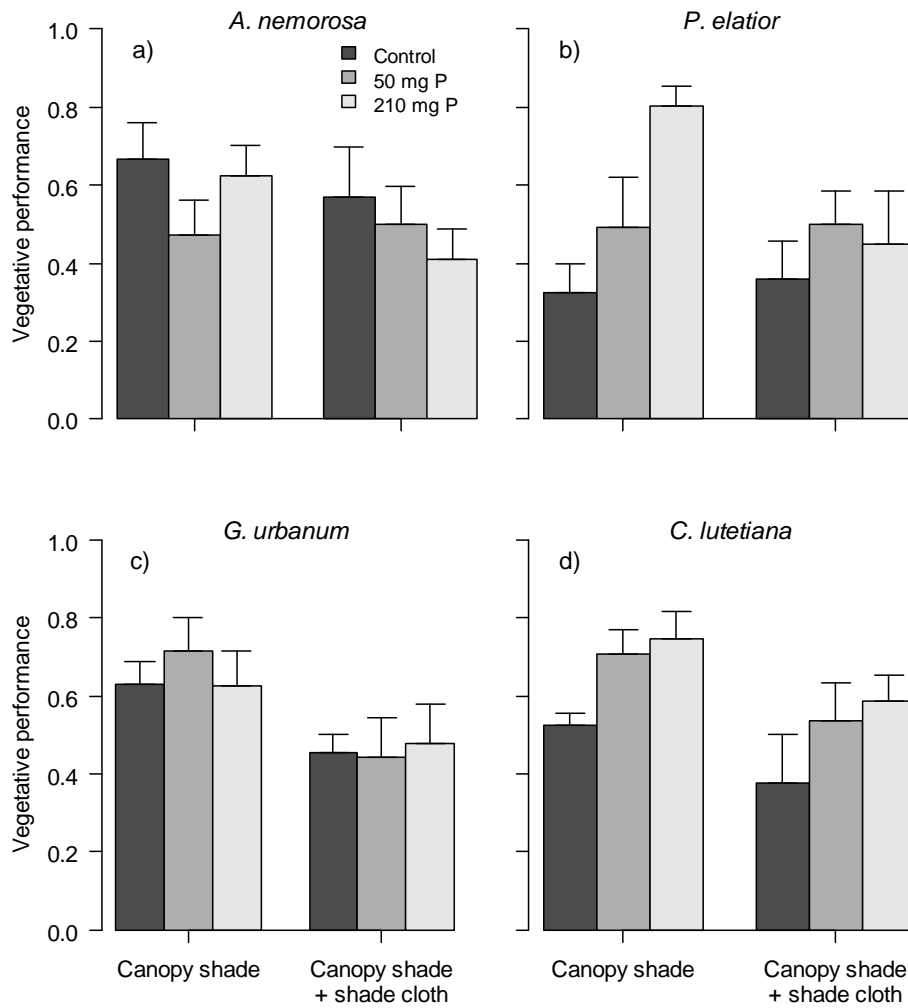


Fig. 2 Effects of P addition and SHADE on the vegetative performance of four forest herbs (mean \pm SE, N = 36 per species). Vegetative performance is the normalized first component of a PCA analysis on vegetative traits. Results of the multilevel models are reported in Table 3a

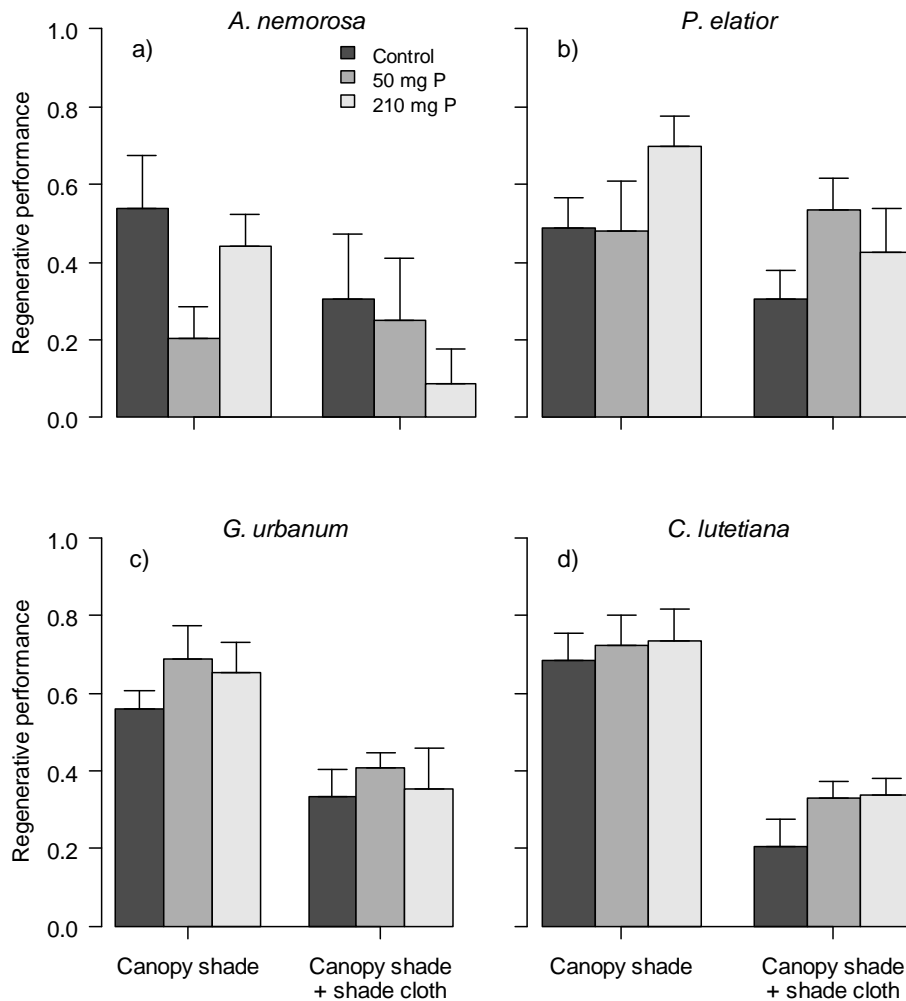


Fig. 3 Effects of P addition and SHADE on the regenerative performance of four forest herbs (mean \pm SE, N = 36 per species). Regenerative performance is the normalized first component of a PCA analysis on regenerative traits. Results of the multilevel models are reported in Table 3b

Discussion

The majority of research on the relationship between herb layer communities and land-use history has produced persuasive evidence that differences in dispersal ability initially control species distributions and abundances in post-agricultural forests (reviewed in Flinn and Vellend 2005; Hermy and Verheyen 2007). After propagule arrival, however, individuals still need to pass through several life-stages before they establish as reproductive adults that contribute to the maintenance of a population (cf. Baeten et al. 2009). The number of studies that explore how the (a)biotic legacies of former agricultural practices influence the establishment, performance and persistence of forest herbs is gradually increasing (e.g., Verheyen and Hermy 2004; Vellend 2005; Fraterrigo et al. 2006; Flinn 2007; Baeten et al. 2009). In the present study we tested whether the persistent P surplus in post-agricultural forests can cause differences in performance among forest herbs and how light availability additionally affects the responses.

The aboveground P concentrations of the four forest herbs in this study all increased in response to the two levels of P addition (Table 2, Fig. 1). The species thus effectively utilized the P surplus and realized a higher P uptake, which indicates physiological plasticity (*sensu* Bradshaw 1965). The increase was typically not proportional to the supply, and in the P treatments, each species maintained concentrations that were well above those of the control, but still below the toxic levels of 1 g P/100 g dry biomass suggested by Marschner (1995). *Circaea lutetiana* turned out to be the most plastic as it concentrated 2.7 times more P in its tissue in the highest P treatment compared to the control. These patterns closely match the results obtained by Rorison (1968, hydroculture) and Hipps et al. (2005, peat-based compost), which partly generalizes their results to more natural settings (i.e., mineral soil, two growing seasons, field conditions). The two summer-flowering species concentrated P more under the shade cloths, but as this was mainly the consequence of a lower biomass, the total aboveground P content did not significantly differ between shaded and unshaded blocks. Anderson (2003) similarly reports higher tissue P concentrations because of lower biomass in shaded plants of the spring ephemeral *Claytonia virginica* L. compared to unshaded plants. The ranges of tissue concentrations in our experiment are also comparable to, or even lower than, the concentrations found in field studies that sampled populations in post-agricultural forest sites in Sweden and Belgium (Falkengren-Grerup et al. 2006; Baeten et al. 2009: *P. elatior* 293 mg P/100 g dry biomass and *G. urbanum* 426 mg P/100 g dry biomass). The applied P levels in our pot experiment thus probably resemble the prevailing pools of bioavailable P under field conditions. Total P content, unlike P concentration, accounts for differences in biomass among individuals and integrates uptake throughout the growing season. Accounting for biomass introduced a considerable proportion of unexplained variation (Table 2b), but the aboveground total P content was still correlated with an increase in P supply. Next to the effects on individual plant performance, which will be discussed below, the accumulation of P into the herbaceous layer might have important consequences for the functioning of post-agricultural forest ecosystems. Because of the rapid decomposition of herbaceous litter, which typically decomposes more than twice as fast as tree leaf litter, the herb layer significantly contributes to the efficiency of the nutrient cycling in forests (Muller 2003; Gilliam 2007). The rapid turnover of P-rich herbaceous litter is likely to be an important process for community dynamics as it directly feeds back upon P availability to plants.

An important objective of this study was to determine whether P availability affects the performance of forest herbs and, if so, whether the effects are related to the capacity of the species to colonize post-agricultural sites (first and third hypothesis). The vegetative performance of two species (*P. elatior*, *C. lutetiana*) was positively related to P availability whereas the performance of the two other species was not (*G. urbanum*) or even slightly negatively (*A. nemorosa*) related to P supply (Table 3a, Fig. 2). Increased P availability as a legacy from former land use may thus directly affect plant performance, but not all species respond in the same way. If different patterns in plasticity among species contribute to differences in their capacity to colonize and persist in post-agricultural forest, i.e., shaping their realized distribution, fast colonizers should respond the most opportunistically to increased P availability (third hypothesis). However, our study does not provide unequivocal evidence for this idea. The two species that increased performance have moderate colonization capacities, while the fast colonizing *G. urbanum* was unaffected. These patterns seem to be partly inconsistent with earlier observations in an eight-year introduction experiment using three corresponding forest herbs (Verheyen and Hermy 2004; Baeten et al. 2009). In this introduction

experiment, *G. urbanum* actually did perform better and persisted in P-enriched post-agricultural sites (cf. Endels et al. 2004), so there must be environmental variables other than P that additionally affect performance (e.g., light availability). On the other hand, *P. elatior* indeed had the intrinsic ability to show higher performance in P-enriched post-agricultural sites (Verheyen and Hermy 2004), but due to indirect effects such as low longevity and competition with opportunistically growing herbs taking advantage of the high P availability (e.g., *Urtica dioica* L.: Pigott and Taylor 1964; Hipps et al. 2005; Taylor 2009), the species could not maintain viable populations (Baeten et al. 2009). Similar indirect effects of P availability, via competition, on the growth of forest herbs were also found by Hipps et al. (2005). Finally, the present pot experiment showed that the spring geophyte *A. nemorosa* was unaffected or even slightly negatively affected by P addition (cf. Verheyen and Hermy 2004; Hipps et al. 2005; Baeten et al. 2009), but the use of soil from two adjacent forest stands introduced a significant amount of variation in both the vegetative and regenerative performance of the species. The two soil types slightly differed in exchangeable cations (mainly K^+ and Mg^{2+}), but other drivers related to land-use history such as the composition of soil biota may equally well account for this effect. Although we must acknowledge that land-use history (post-agricultural versus ancient forest soil) was not truly replicated, it is a remarkable result that *A. nemorosa* performed better in the post-agricultural soil, which was not P enriched, but tended to be negatively affected by additional P supply. Further study is required to unravel the complex effects – which seem to be beneficial as well as detrimental – of former land-use on the growth of similar forest herbs.

While the regenerative performance was largely unaffected by the P treatment, light availability had a major impact on the regenerative performance of the two summer-flowering herbs (Table 3b, Fig. 3). The number and height of inflorescences and the number of flowers (*G. urbanum*) or flowering frequency (*C. lutetiana*) were reduced in experimental blocks that were covered with a shade cloth. In an allocation study, Verburg and Grava (1998) also found positive effects of light availability on the reproductive effort of *C. lutetiana*. They showed that fruit production was size dependent, which is consistent with the positive relationship we found between vegetative and regenerative performance of the species. Similarly, in an ecophysiological study, Pons (1977) showed that *G. urbanum* performed better in terms of biomass in high light compared to low light conditions. The dominant effect of light availability on the performance of *G. urbanum* and the lack of response to P addition at least suggests that the higher performance of the species in post-agricultural compared to ancient forest sites (Endels et al. 2004; Verheyen and Hermy 2004; Baeten et al. 2009) is partly the result of differences in light conditions. The spring-flowering species avoid canopy shade through phenological escape and were not affected by the shade treatment. Since we only measured a suite of morphological traits to determine the extent of regenerative performance, it remains to be studied how other components of reproductive output such as the number of seeds, seed mass and germination are affected by former land use. Based on previous experimental work on the effects of nutrient and light availability on the growth of forest herbs (e.g., Meekins and McCarthy 2000; Elemans 2004), we expected interactive effects of P and SHADE on P uptake and plant performance (second hypothesis). Experiments with the spring ephemeral *C. virginica* in a deciduous forest in Tennessee, for instance, showed that NPK fertilization increased the vegetative and reproductive biomass of plants growing at ambient sunlight more than for plants growing under experimental shade treatments (Eickmeier and Schlusser 1993; Anderson 2003). It was suggested that shaded plants have a lower demand for nutrients because low light constrains their growth so that the plants may not be able to respond to increased nutrient availability at lower light levels. In our study, the

interactive effects of P and SHADE on the aboveground P concentration (*C. lutetiana*) and total P content (*P. elatior*) also indicated that shading may partly mask the response of forest herbs to higher nutrient availability. For the performance measures, we did not find significant interaction terms in the multilevel models, but synergistic effects of P and SHADE on the overall plant performance did emerge. For *C. lutetiana*, vegetative traits were positively related to the addition of P whereas light mainly affected regenerative traits. So, the overall performance in this species obviously depended on the availability of both resources.

We used four herb species with differing combinations of life form and phenology to uncover response patterns related to these life-history characteristics. Although light differentially affected the spring and summer-flowering species, the effect of P availability was unrelated to life form and phenology. This result suggests that responses to P availability are largely species specific, or related to other life-history attributes, which turns it difficult to make general predictions about other species. Similarly, Fratterigo et al. (2006) found species-specific effects of land-use history on the growth and performance of twelve forest herbs in southern Appalachian forests. Because it remains unclear which traits or trait combinations related to performance are promoted by former agricultural land use, and more specifically P availability, it is difficult to predict how P exactly contributes to the structuring of herbaceous communities in post-agricultural forest. The potential effects of P availability on forest communities should also be considered in relation to other key resources. A persistent P surplus might, for instance, provide the increasing demand for P that is expected at high levels of chronic N deposition (Gress et al. 2007). This would clear the way for highly productive forest vegetations in which forest herbs that have the ability to benefit from P (e.g., *P. elatior*, *C. lutetiana*) are still competitively excluded.

To conclude, the persistently higher P availability in post-agricultural forests may have positive, species-specific effects on the vegetative performance of forest herbs (cf. first hypothesis; Rorison 1968; Hips et al. 2005). The regenerative performance, on the other hand, was mostly related to light availability. It is important to notice, however, that pot experiments only determine the potential plastic response of individuals and ignore complex community interactions. Comparing our results with extensive field observations (cf. Baeten et al. 2009) at least suggests that indirect effects such as competition may cancel, if not reverse, the effects of P availability on population persistence in herbaceous communities. *Primula elatior* proved to be an informative case as it benefits from P addition, but, simultaneously, suffers from competition with P-limited competitors in field conditions (Baeten et al. 2009). Addressing how differences in the plastic response among forest herbs alter the magnitude of interactions between species (trait-mediated interactions *sensu* Callaway et al. 2003) and structure forest plant communities is an important direction for future research. Furthermore, land-use history not only influences the distribution and abundance of forest herb populations, it also impacts their genetic diversity. Vellend (2004), for instance, found reduced genetic diversity and genetic divergence (drift) in *Trillium grandiflorum* (Michx.) Salisb. populations in secondary forests via land-use effects on effective population size. Jacquemyn et al. (2009) showed a rapid decline in genetic diversity in a founding population of *P. elatior* colonizing recent forest stands. We therefore argue to perform similar experiments such as the present on individuals from different populations (e.g., populations in ancient versus post-agricultural forest) and genetically distinct groups to explore how genetic variability controls the plasticity of forest herbs and to gain further insights into the complex effects of land-use history on forest plant populations.

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