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Soil phosphorus constrains biodiversity across European grasslands

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Abstract

Nutrient pollution presents a serious threat to biodiversity conservation. In terrestrial ecosystems, the deleterious effects of nitrogen pollution are increasingly understood and several mitigating environmental policies have been developed. Compared to nitrogen, the effects of increased phosphorus have received far less attention, although some studies have indicated that phosphorus pollution may be detrimental for biodiversity as well. On the basis of a data-set covering 501 grassland plots throughout Europe, we demonstrate that, independent of the level of atmospheric nitrogen deposition and soil acidity, plant species richness was consistently negatively related to soil phosphorus. We also identified thresholds in soil phosphorus above which biodiversity appears to remain at a constant low level. Our results indicate that nutrient management policies biased toward reducing nitrogen pollution will fail to preserve biodiversity. As soil phosphorus is known to be extremely persistent and we found no evidence for a critical threshold below which no environmental harm is expected, we suggest that agro-environmental schemes should include grasslands that are permanently free from phosphorus fertilization.

Keywords: atmospheric nitrogen deposition, environmental policy, grassland, nutrient enrichment, phosphorus

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Introduction

Since the beginning of the industrial revolution, anthropogenic activities have caused an unprecedented input of nitrogen (N) and phosphorus (P) in the biosphere (from 15.3 to 259 Tg N yr^{-1} and <0.3 to 16 Tg P yr^{-1}; Peñuelas *et al.*, 2012). Increased nitrogen flows originate primarily from the combustion of fossil fuels and the use of industrial fertilizers manufactured from airborne N_2 via the Haber-Bosh procedure. Increased phosphorus flows on the other hand, originate mainly from the application of mineral fertilizers from mined rock-reserves and the use of livestock slurry and manure (Vance *et al.*, 2002; Peñuelas *et al.*, 2012, 2013). Excess agricultural fertilizer can subsequently be redistributed into adjacent ecosystems via runoff or transport via freshwater bodies. Atmospheric deposition of volatilized nitrogenous compounds and, to a lesser extent, mineral aerosols of dust from phosphorus fertilizers, further contribute to nutrient pollution of natural ecosystems (Newman, 1995; Peñuelas *et al.*, 2013). Finally, anthropogenic changes to the soil biogeochemical conditions that affect soil phosphorus sequestration, such as sulfurous pollution, can disrupt natural phosphorus balances leading to increased soil phosphorus

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availability (Addiscot and Thomas 2000, Hinsinger 2001, Turner & Haygarth, 2001).

The consequences of increased nutrient flows in the biosphere have become one of the major components of global environmental change (Sala *et al.* 2000, Foley *et al*., 2005; Galloway *et al*., 2008). Indeed, a large number of studies identify increased nitrogen inputs, particularly via atmospheric nitrogen deposition, as a major culprit of biodiversity loss in both terrestrial and aquatic ecosystems worldwide (e.g., Stevens *et al*., 2004; Phoenix *et al*., 2006; Clark & Tilman, 2008; Conley *et al*., 2009; Cleland & Harpole, 2010). Particular attention has been paid to the industrialized parts of the world, as the atmospheric nitrogen deposition here may exceed more than a 10-fold of the expected natural background deposition (~5 kg N ha^{-1} yr^{-1}; Galloway *et al*., 2008). To mitigate the deleterious impact of nitrogen pollution, a suite of nitrogen management policies have been developed and attempts have been made to identify critical loads of nitrogen deposition, below which there should be no adverse effects on biodiversity (Bobbink *et al*., 2010; Sutton *et al*., 2011; Payne *et al*., 2012).

Similar to nitrogen, there is a risk of phosphorus pollution in the industrialized countries, owing to the high agricultural application of phosphorus fertilizer, frequently associated with intensive livestock production (Reijneveld *et al*., 2010; Obersteiner *et al*., 2013). For instance, 47% of the tested agricultural soils in New York state and over 50% of agricultural soils in the Netherlands, Sweden and Belgium showed soil phosphorus levels well above the recommended ranges (Djodjic *et al*., 2004; BDB, 2005; Ketterings *et al*., 2005; Reijneveld *et al*., 2010). Compared to nitrogen pollution, the environmental consequences of increased phosphorus have received far less attention (Elser & Bennet, 2011). Furthermore, environmental policies aimed at mitigating phosphorus pollution have mainly targeted aquatic ecosystems (e.g. Schindler *et al*., 2008; Conley *et al*., 2009). Nevertheless, some studies have indicated that phosphorus pollution may be at least equally detrimental for biodiversity in terrestrial ecosystems, independent of the level of nitrogen pollution (Wassen *et al*., 2005; Ceulemans *et al*., 2013; Fujita *et al*., 2013). To develop appropriate environmental policies with respect to nutrient enrichment in terrestrial ecosystems, there is a need to disentangle the contribution of nitrogen and phosphorus pollution to biodiversity loss.

In this study, we aimed at identifying the relationships between nitrogen and phosphorus on the one hand and plant species richness in European grasslands on the other. It has been established that European grasslands are susceptible to increased nitrogen deposition (e.g., Stevens *et al*., 2010), but owing to agricultural intensification, particularly live stock production (Reijneveld *et al*., 2010), they may also be at risk of phosphorus enrichment. This is one of the first large scale studies that directly and simultaneously takes indicators of both nitrogen and phosphorus pollution into account when investigating species diversity. Furthermore, we tested whether the observed relationships differ between different grassland types or could be generalized. Finally, to provide tools for developing environmental policies, we aimed at identifying possible critical thresholds in soil phosphorus levels with respect to biodiversity.

Materials and methods

Data collection

We surveyed three common grassland types in Europe (314 *Nardus* grasslands in 10 European countries and 105 lowland hay meadows and 82 calcareous grasslands spread across five European countries; Fig. 1). These grasslands are often components of agricultural landscapes, providing grazing areas for cattle and sheep (Veen *et al.* 2009). Species rich examples of these three grassland communities are protected by the European Habitat Directive, the European legislative

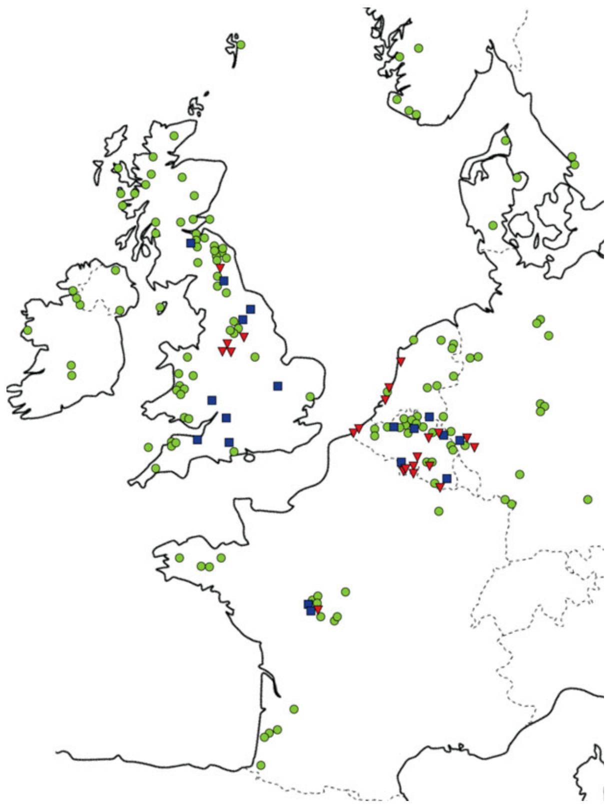

Fig. 1 Map of the European regions where grassland quadrats were surveyed (N = 501). Nardus grasslands: green, N = 314; lowland hay meadows: blue, N = 105 and calcareous grasslands: red, N = 82.

instrument for the conservation of wild animal and plant species and natural habitats of community importance (*Nardus* grasslands: Habitat type 6230, lowland hay meadows: Habitat type 6510 and calcareous grasslands, Habitat types 6210 and 2130). For the purposes of this research, we compiled a dataset consisting of two methodologically similar datasets collected to investigate the effects of nutrient pollution on species composition and richness of *Nardus* grasslands (Stevens *et al.* 2011, $N = 153$ and Ceulemans *et al.*, 2011, $N = 132$). Using the same methodology, we additionally surveyed 29 *Nardus* grasslands, 105 lowland hay meadows and 82 calcareous grasslands. As *Nardus* grasslands were subject of two previous studies with a larger time span, sampling of lowland hay meadows and calcareous grasslands did not cover the same spatial range as *Nardus* grasslands. Based on their high similarity in species composition, calcareous Atlantic dune grasslands were lumped with inland calcareous grasslands. Access permission for all the sites was gained through landowners and responsible agencies for protected areas. Grasslands were surveyed from late May to early August, between 2002 and 2013.

To ensure consistent habitat selection, surveyed grasslands needed to meet three main criteria. Firstly, we drew up lists of indicative plant species for all three grassland types, based on the Interpretation Manual of European Union Habitats (European Commission, 2007). We only sampled grasslands meeting the general description of the habitat type and containing at least four indicative species (Table S1). Secondly, to avoid extinction debt, we only surveyed grasslands that did not receive direct agricultural fertilizer application in the recent past. Nevertheless, nutrient enrichment of these grasslands may still occur through inflow from adjacent arable land (*Nardus* and calcareous grasslands on slopes), via polluted freshwater bodies (lowland hay meadows in valleys) or via redistribution by livestock. Finally, to minimize possible effects of agricultural intensification on biodiversity other than nutrient enrichment, surveyed grassland were not ploughed or reseeded and received continuous extensive management by cutting or cutting and grazing. To control for the latter two criteria, we inquired for site management history dating back at least 17 years prior to sampling. We then selected areas in Europe in a stratified manner to cover as much of the typical range of atmospheric nitrogen deposition as possible (Fig. 1). Based on information from national datasets, vegetation maps and local conservation agencies, we randomly chose grassland sites in these areas meeting the above mentioned selection criteria.

Each grassland site was surveyed for plant species presence before establishing a quadrat of 2 m by 2 m containing as much of the observed dominant and characteristic plant species of the site as possible. For a subset of the data (*Nardus* grasslands $N = 153$ and lowland hay meadows $N = 105$), between three and five randomly placed replicate quadrats within a 1-ha sampling area of the desired grassland type were placed. To give one record per site, the median species richness and mean of the environmental variables were calculated. In each quadrat, we recorded plant species richness and then took between two and ten top-soil samples (0–10 cm, on shallower soils as deep as possible) with an auger (2–5 cm diameter). These samples were then air-dried, stones and roots were removed, and the samples were thoroughly homogenized prior to soil analyses.

Soil phosphorus was determined using the Olsen-P extraction and subsequent colorimetrical analysis using the molybdenum blue method (Robertson *et al.*, 1999). Although this method has been identified as the most suitable extraction method to assess plant available phosphorus (Gilbert *et al.*, 2009), we also performed soil phosphorus extraction on a subset of the quadrats with anion exchange membranes and oxalic acid extraction to compare the results ($N = 84$ and $N = 36$ respectively; Robertson *et al.*, 1999). We found very high Pearson correlations across the entire pH range and similar relationships with plant species richness across all three phosphorus extraction methods (Figure S1). Although we used soil extractable phosphorus as indicator for phosphorus enrichment in this study, some of the observed variation in soil phosphorus levels may be attributed to inherent and natural differences in soil mineralogy. Nevertheless, reports of soil phosphorus levels in unfertilized or unimproved similar grassland communities vary from 4 mg P kg^{-1} to 25 mg P kg^{-1} (Olsen-P method; Critchley *et al.*, 2002; Silvertown *et al.*, 2006; Gilbert *et al.*, 2009), and levels above 20–30 mg P kg^{-1} are considered to be an indication of anthropogenic phosphorus enrichment (Critchley *et al.*, 2002). It is therefore likely that most of these elevated levels of soil phosphorus in our study represent a gradient in phosphorus enrichment.

As indicator for nitrogen pollution, we obtained data on total atmospheric nitrogen deposition, consisting of wet and dry deposition of oxidized and reduced nitrogen, from the best available local estimation model. National models were used for Germany (Gauger, 2002), the Netherlands (Asman & van Jaarsveld, 2002), Great Britain (NEGTAP, 2001) and the northern part of Belgium (Flanders, VMM, 2010). For all other countries the EMEP-based IDEM models were used (Pieterse *et al.*, 2007). The different models use similar approaches to model nitrogen deposition. For all of the models, deposition was calculated as a three-year average to provide a more robust estimate of long-term nitrogen inputs (years 2000–2002). We did not determine soil nitrogen for all quadrats, as it does not always provide a good approximation of plant available nitrogen (1 M KCl method, Robertson *et al.*, 1999). It proved unfeasible to determine nitrogen mineralization rates, which provides a better measure, on all 501 grasslands. Nevertheless, we report the results including soil nitrogen for a subset of the data in supplementary information indicating no significant relationships with soil phosphorus or plant species richness (Table S2; $N = 283$).

Finally, we also determined soil acidity using a pH glass electrode in a soil-water mix that was shaken for 30 min (soil/distilled water: 1/2.5).

Statistical analyses

First we calculated Pearson correlations between soil phosphorus, soil pH and total nitrogen deposition to check for possible relationships between the explanatory variables. Next, as species richness is a count variable, Poisson regression models were used to investigate the relationship between soil phosphorus, soil pH and total nitrogen deposition on the one side and species richness on the other. To account for differences in geography and climate, we also included longitude and latitude of each quadrat as explanatory variables. Grassland type was used as a categorical variable to account for differences in species pools and species composition between the grassland types. We included all first order interactions between the explanatory variables in a first full factorial model. Next, the interaction factors were one by one removed to build a suite of reduced models (the fully reduced model contained the six main variables mentioned above). Out of the models whit all possible combinations of interaction factors, we selected the most parsimonious model using the Akaike Information Criterion (AIC). As grassland quadrats were sampled over a long time period, we also checked whether year of sampling may influence our analyses. We found no significant effect of year of sampling on plant species richness and including year of sampling as variable did not change any of the other relationships we found in this study (Table S3).

To identify possible critical thresholds in soil phosphorus, piecewise Poisson regression analyses were performed for each grassland type separately, with soil phosphorus as explanatory variable and species richness as response variable. In these analyses, possible thresholds were identified based on a significant nonlinear change in the response of plant species richness to soil phosphorus and were estimated by a 'breakpoint' or 'knot' connecting two regression lines (Toms & Lesperance, 2003). We built three different piecewise regression models to allow for different possible responses of species richness to soil phosphorus. In the first model, both regressions were allowed to follow a linear path according to the equations (a):

$$\text{(i) Ln(Plant species richness)} = \beta_0 + \beta_1{}^*\text{soilP}$$
$$\text{for soilP} \leq \text{knot (intercept} = \beta_0; \text{slope} = \beta_1)$$
$$\text{(ii) Ln(Plant species richness)} = (\beta_0 - \beta_2{}^*\text{knot}) + (\beta_1 + \beta_2)^*\text{soilP}$$
$$\text{for soilP} \geq \text{knot (intercept} = \beta_0 - \beta_2{}^*\text{knot}; \text{slope} = \beta_1 + \beta_2)$$

in the second model, the first regression was modeled as a constant and the second regression was allowed a linear path according to the equations (b):

$$\text{(i) Ln(Plant species richness)} = \beta_0$$
$$\text{for soilP} \leq \text{knot (intercept} = \beta_0; \text{slope} = 0)$$
$$\text{(ii) Ln(Plant species richness)} = \beta_0 + \beta_1{}^*(\text{soilP} - \text{knot})$$
$$\text{for soilP} \geq \text{knot (intercept} = \beta_0; \text{slope} = \beta_1)$$

and in the third model the first regression was allowed a linear path and the second regression was modeled as a constant according to the equations (c):

$$\text{(i) Ln(Plant species richness)} = \beta_0 + \beta_1{}^*\text{soilP}$$
$$\text{for soilP} \geq \text{knot (intercept} = \beta_0; \text{slope} = \beta_1)$$
$$\text{(ii) Ln(Plant species richness)} = \beta_0 + \beta_1{}^*\text{knot}$$
$$\text{for soilP} \leq \text{knot (intercept} = \beta_0 + \beta_1{}^*\text{knot}; \text{slope} = 0)$$

Note that the latter two sets of equations are special cases of the first set where β_0 equals 0 and $-\beta_1$ respectively. All regression models calculated the results based on maximizing the likelihood of all parameters (two possible slopes and a break point). Out of the three piecewise regression models and a single linear Poisson regression (without a breakpoint), the model that best described the data was selected based on the lowest AIC value. The SAS programs used to perform the piecewise regression analyses are included in supporting information.

Results

A total of 571 plant species were found in the 501 grassland quadrats. Frequent species in *Nardus* grasslands were *Agrostis capillaris*, *Danthonia decumbens*, *Nardus stricta*, *Molinia caerulea*, *Potentilla erecta*, *Succisa pratensis* and *Galium saxatile*. In lowland hay meadows frequent species were *Holcus lanatus*, *Alopecurus pratensis*, *Cynosurus cristatus*, *Cardamine pratensis* and *Sanguisorba officinalis*. Frequent species in calcareous grasslands included *Brachypodium pinnatum*, *Bromus erectus*, *Koeleria pyramidata* s.l., *Carex flacca*, *Sanguisorba minor*, *Teucrium chamaedrys*, *Ononis repens* and *Helianthemum nummularium*. Details of the environmental variables used in the Poisson regression models can be found in Table 1.

Invariably, the highest species richness occurred at lower levels of soil phosphorus. Maximum observed species richness did not exceed 20 species per 4 m^2 beyond 80 mg P kg^{-1}, compared to more than 40 species below 40 mg P kg^{-1} (Fig. 2). The Poisson regression analysis with the lowest AIC showed a highly significant negative relationship between soil phosphorus and species richness across all grassland types (Wald $\chi 2 = 208.36$, $P < 0.0001$, Table 2). This negative relationship was independent of nitrogen deposition and soil pH as we found no significant Pearson correlations between soil phosphorus and nitrogen deposition or soil pH ($r = -0.02$, $P = 0.63$; $r = 0.01$, $P = 0.77$ respectively, Figure S2) and no significant interactions with soil phosphorus (Table 2). Nitrogen deposition and soil pH were also significantly negatively related to species richness, reflected by the only remaining interaction term in the model showing a gradually stronger negative relationship between nitrogen deposition and species richness in more acidic sites (Wald $\chi 2 = 57.48$, $P < 0.0001$, Table 2). In addition, we found that lowland hay meadows had significantly lower species richness than calcareous grasslands and *Nardus* grasslands (Wald $\chi 2 = 83.7$, $P < 0.0001$, Table 2). Geographical location was not significantly related to species richness (Table 2).

Piecewise regression analyses with the lowest AIC's invariably showed that the relationship between soil phosphorus and plant species richness was best described by an initial log-linear decrease in species richness until a 'threshold' in soil phosphorus has been

Table 1 Details of variables used in the Poisson regression models. Unit for species number is: count 4 m^{-2}; soil phosphorus (Olsen method): mg kg^{-1} and total nitrogen deposition: kg ha^{-1} yr^{-1}

Variable	Mean	Range	Mean	Range
	All grasslands (N = 501)		Hay meadows (N = 105)	
Species number	21.57	4–49	17.28	5–31
Soil P	31.82	0–305.45	28.17	0–266.48
Soil pH	5.44	3.69–8.27	5.58	4.26–7.93
Total N deposition	18.50	2.3–43.5	21.50	10.9–27.8
	Calc. grasslands (N = 82)		*Nardus* grasslands (N = 314)	
	Mean	Range	Mean	Range
Species number	24.44	12–40	22.25	4–49
Soil P	38.01	0.22–200.33	31.45	0–305.45
Soil pH	6.90	4.83–8.27	5.01	3.69–7.37
Total N deposition	18.02	10.19–25	17.6	2.3–43.5

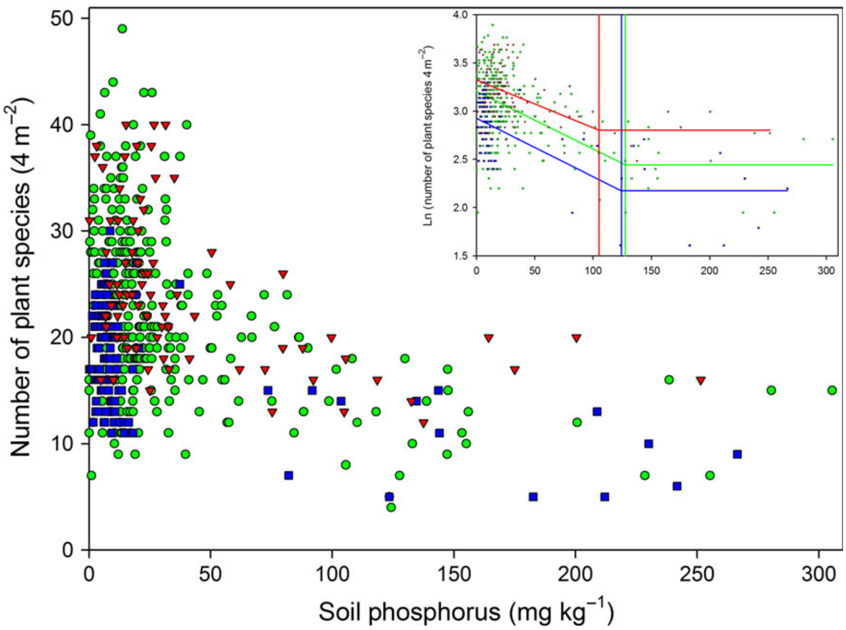

Fig. 2 Relationship between soil phosphorus and plant species richness across three types of European grasslands (N = 501). Nardus grasslands: green, N = 314; lowland hay meadows: blue, N = 105 and calcareous grasslands: red, N = 82. The inset depicts the piecewise Poisson regression models with breakpoints (vertical reference lines depict the breakpoints, see Table 3).

reached, after which species richness remained at a constant level (Fig. 2). In *Nardus* grasslands, no further decreases in species richness were observed beyond 127.6 mg P kg^{-1}, remaining at an average of 12.5 species per quadrat. In calcareous grasslands, species richness stabilized at 17.2 species beyond 104.9 mg P kg^{-1} and in lowland hay meadows at 9.8 species beyond 124.3 mg P kg^{-1} (Fig. 2; Table 3).

Discussion

Our results suggest that, independent of the environmental impacts of nitrogen pollution, soil phosphorus constrains plant species richness in European grasslands. Indeed, we found no relationship between soil phosphorus on the one hand and nitrogen deposition on the other. Furthermore, at any level of nitrogen deposition or soil pH, quadrats with high soil phosphorus consistently showed lower species richness. This was not restricted to a specific grassland community, as average species richness dropped at similar rates across all three grassland types. Although we cannot infer a direct causal relationship, our observations are consistent with grassland fertilization experiments indicating long-lasting negative effects of phosphorus amendment on plant species richness and characteristic plant

Table 2 Detailed results of the Poisson regression model with lowest AIC examining the relationships between soil phosphorus, soil pH, total nitrogen deposition, longitude, latitude and grassland type and plant species richness (N = 501). For interpretation of the interaction between nitrogen deposition and soil pH, estimated slopes at shifted intercepts of nitrogen deposition and soil pH are included. Shifted intercepts are: $^{1}pH_{observed}$-3, $^{2}pH_{observed}$-5, $^{3}pH_{observed}$-6.5 and $^{a}Ndep_{observed}$-10, $^{b}Ndep_{observed}$-20, $^{c}Ndep_{observed}$-30. We shifted the intercepts by subtracting 3, 5 and 6.5 respectively from all observed pH values and then recalculated the estimated slopes of total N deposition (vice versa for Ndep). This was necessary as the default intercept corresponds to pH = 0 or Ndep = 0, which is ecologically senseless. Unit for species number is: ln(count) 4 m^{-2}; soil phosphorus: mg kg^{-1} and total nitrogen deposition: kg ha^{-1} yr^{-1}. δAIC compared with the fully reduced model without interaction factor is 56.6

	Estimate	Standard Error	Wald χ^2	P-value
Intercept	4.570	0.255	322.29	<0.0001
Soil phosphorus	−0.004	0.000	208.36	<0.0001
Total N deposition *Soil pH	0.012	0.002	57.48	<0.0001
Total N deposition	−0.075	0.008	85.51	<0.0001
Soil pH	−0.148	0.031	22.53	<0.0001
Latitude	−0.006	0.003	3.80	0.05
Longitude	0.001	0.002	0.24	0.67
type = Lowland hay meadows	−0.288	0.032	83.70	<0.0001
type = Calcareous grasslands	−0.004	0.038	0.01	0.92
type = *Nardus* grasslands	0	0	.	.
Total N deposition[1]	−0.040	0.004	116.96	<0.0001
Total N deposition[2]	−0.016	0.002	22.53	<0.0001
Total N deposition[3]	0.002	0.003	0.53	0.47
Soil pH[a]	−0.029	0.019	2.35	0.13
Soil pH[b]	0.090	0.015	34.90	<0.0001
Soil pH[c]	0.209	0.025	72.58	<0.0001

species (Crawley *et al.*, 2005; Hejcman *et al.*, 2005, 2010); as well as with local observations of lower species richness in grasslands with higher phosphorus availability (Janssens *et al.*, 1998; Härdtle *et al.*, 2006; Kleijn & Müller-Schärer, 2006). Nevertheless, the results of this study do not contradict findings of negative effects of nitrogen pollution on biodiversity, particularly through high rates of atmospheric nitrogen deposition (e.g., Stevens *et al.*, 2004; Cleland & Harpole, 2010). Indeed, we found a clear negative relationship between atmospheric nitrogen deposition and plant species richness, mediated by soil pH, suggesting a gradually higher loss of plant species richness in more acidic grasslands. This supports a suite of studies demonstrating that the negative effects of nitrogen deposition on biodiversity are primarily connected with soil acidification (e.g. Horswill *et al.*, 2008; Stevens *et al.*, 2010).

According to our results, we can expect that current nutrient management policies in terrestrial ecosystems, primarily biased toward reducing nitrogen pollution, will fail to preserve biodiversity. A key policy tool for mitigating nitrogen pollution has been the critical load of nitrogen input, calculated based on fertilization experiments and observations along gradients of nitrogen pollution (Sutton *et al.*, 2011; Payne *et al.*, 2012). A critical load indicates the level of pollution that can be tolerated by an ecosystem without harmful effects. In this study, we aimed at identifying similar critical loads for levels of soil phosphorus. We found indications that loss of plant species richness following phosphorus pollution may only occur below certain soil phosphorus levels. Indeed, average species richness in all three grassland types remained at a constant low level in quadrats with soil phosphorus exceeding 104-130 mg P kg^{-1} (Fig. 2, Table 3). However, these thresholds do not show a critical level below which no harm on biodiversity is to be expected, but indicate that the least harm to biodiversity can be expected when no phosphorus pollution takes place. A drawback of our dataset is the relatively poor representation of grasslands with high soil phosphorus levels. This is due to site selection criteria demanding no sites under direct fertilization and which is based on the presence of characteristic plant species that may already have been lost at these levels of soil phosphorus (e.g. Ceulemans *et al.*, 2011). Nevertheless, combined with the general log-linear relationship between soil phosphorus and plant species richness, these thresholds suggest that the largest loss of biodiversity may already occur at small rates of phosphorus enrichment. Therefore, and consistent with recent suggestions regarding nitrogen pollution (Payne *et al.*, 2012), it seems necessary that environmental policy decisions are based on a sliding scale of phosphorus pollution, rather than on critical loads that assume no harm below certain levels of pollution.

The strong negative relationship between soil phosphorus and plant species richness, similar across the three different grassland types, suggests particular ecological mechanisms determining this plant species loss. Loss of plant diversity following nutrient enrichment may be caused by increased productivity and subsequent competitive exclusion of species (Hautier *et al.*, 2009). However, the impact of increased productivity is likely to be weak as previous work on a subset of our grasslands showed only a small effect on species richness, and enhanced productivity following increased soil phosphorus was primarily restricted to phosphorus limited grasslands (Ceulemans *et al.*, 2013). Alternatively, it has been hypothesized that the large

Table 3 Detailed results of the piecewise Poisson regression models with lowest AIC examining a possible threshold (knot) of soil phosphorus with respect to plant species richness per grassland type. Unit for species number is: Ln(count) 4 m^{-2} and soil phosphorus: mg kg^{-1}. δAIC compared with a single regression model without breakpoint was 4.7 for lowland hay meadows, 4.0 for calcareous grasslands and 10.2 for *Nardus* grasslands

	Estimate	Standard error	*t* Value	*P*-value	DF
Lowland hay meadows					
Intercept	2.952	0.029	103.10	<0.0001	105
Slope	−0.005	0.001	−3.70	<0.0001	105
Knot	124.28	36.80	3.38	<0.001	105
Calcareous grasslands					
Intercept	3.350	0.033	102.51	<0.0001	82
Slope	−0.005	0.001	−6.04	<0.0001	82
Knot	104.86	0.049	2119.52	<0.0001	82
Nardus grasslands					
Intercept	3.250	0.016	200.58	<0.0001	314
Slope	−0.006	0.000	−12.37	<0.0001	314
Knot	127.64	0.04	2898.11	<0.0001	314

variety of chemical phosphorus compounds in the soil (complexes with Ca, Fe, Mg, Al or organic compounds) may facilitate high resource partitioning for phosphorus uptake and subsequently promote species coexistence in phosphorus poor soils (Turner, 2008; Olde Venterink, 2011). In this context, it is also noteworthy that mycotrophic species, which are able to tap into phosphorus pools otherwise unavailable for plant uptake through their mycorrhizal partner, appear susceptible to increased soil phosphorus (Ceulemans *et al*., 2011). However, although resource partitioning has been demonstrated for uptake of nitrogen compounds (McKane *et al*., 2002), ecologists have yet to demonstrate this with respect to phosphorus uptake.

Based on this study, it appears that both nutrients may contribute to biodiversity loss in terrestrial ecosystems. However, anthropogenic nutrient enrichment tends to fertilize the biosphere with phosphorus more locally and at a smaller rate than nitrogen (Peñuelas *et al*., 2012). Indeed, nitrogen pollution originates mainly from seemingly unlimited sources (fossil fuels, industrial fertilizers manufactured from airborne N_2 via the Haber-Bosh procedure), whereas phosphorus pollution originates mainly from the application of fertilizer manufactured from dwindling rock-reserves (Vance *et al*., 2002; Peñuelas *et al*., 2012, 2013). Furthermore, both airborne nitrogenous compounds and soil nitrogen are more mobile than soil phosphorus compounds or mineral phosphorus aerosols (Gough & Marrs, 1990; Newman, 1995; Peñuelas *et al*., 2012). Therefore, terrestrial ecosystems in or near agricultural areas are the most likely to suffer from phosphorus pollution, indirectly through runoff from nearby arable land or mineral aerosol deposition or directly through phosphorus fertilization (Newman, 1995; Peñuelas *et al*., 2012). Important in this context is that phosphorus fertilizer recommendations for grasslands used to produce live stock frequently exceed the apparent phosphorus thresholds we identified (~100–130 mg P kg^{-1}; e.g., Reijneveld *et al*., 2010). In addition, phosphorus fertilization causes chronically enhanced soil phosphorus owing to tight sequestration in the soil (Sattari *et al*., 2012). Consequently, even low levels of phosphorus inputs can be damaging in the long term through cumulative effects. Therefore, high biodiversity cannot be expected in fertilized sites in the foreseeable future, even after implementing agro-environmental schemes reducing phosphorus fertilization in favor of nature conservation.

To conclude, and despite our support for efforts to reduce nitrogen pollution (e.g. Phoenix *et al*., 2006), we highlight the need to develop a global perspective regarding the effects of phosphorus pollution on terrestrial biodiversity. Noteworthy in this context is that the negative impact of soil phosphorus on biodiversity, may not be exclusively restricted to Europe. Indeed, two studies indicated that plant species richness and the occurrence of characteristic plant species is restricted by high soil phosphorus in both Australian savanna and in Northeast American pastures (Tracy & Sanderson 2000, Dorrough & Scroggie, 2008). Finally, based on the long term enhanced soil phosphorus following fertilization and the lack of a critical threshold below which no environmental harm is expected, we strongly advocate that agro-environmental schemes should include grasslands permanently free from phosphorus fertilization.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationships between the different phosphorus extraction methods and plant species richness (N = 84 for Olson-P and AEM-P, N = 36 for Oxalic acid-P). Red triangles represent grassland quadrats with pH < 5.5, blue circles 5.5 < pH < 7, green squares pH > 7. Respective Pearson correlation coefficients are from top left to top right: $r = -0.56$, $P < 0.001$; $r = -0.59$, $P < 0.001$; $r = -0.60$, $P < 0.001$ and from bottom left to bottom right: $r = 0.81$, $P < 0.001$; $r = 0.78$, $P < 0.001$; $r = 0.80$, $P < 0.001$.
Figure S2. Relationship between plant species richness, soil phosphorus and total nitrogen deposition across three types of European grasslands. Plane equation: ln(Plant species richness) = 3.53 − 0.004(soil P) − 0.022(N deposition). *Nardus* grasslands: green, N = 314; lowland hay meadows: blue, N = 105 and calcareous grasslands: red, N = 82. We found no significant Pearson correlation between soil phosphorus and nitrogen deposition ($r = -0.02$, $P = 0.63$).
Table S1. List of the indicative species that were used to identify the surveyed grassland types.
Table S2. Detailed results of the Poisson regression model examining the relationships between soil phosphorus, soil pH, total nitrogen deposition, longitude, latitude, grassland type and soil nitrogen (1 M KCl extraction, $NH_4^+ + NO_3^-$) and plant species richness (N = 293). Including soil nitrogen did not change the results of our general analyses (Table 2) and had no significant effect on plant species richness. Soil nitrogen showed significant Pearson correlation coefficients with total nitrogen deposition ($r = 0.18$, $P = 0.02$) and soil pH ($r = -0.35$, $P < 0.001$) but not with soil phosphorus ($r = -0.1$, $P = 0.09$).
Table S3. Detailed results of the Poisson regression model examining the relationships between soil phosphorus, soil pH, total nitrogen deposition, longitude, latitude, grassland type and year of sampling and plant species richness (N = 490). Year of sampling did not change the results of our general analyses (Table 2) and had no significant effect on plant species richness. For 11 quadrats we had no data on the year of sampling.