Determinants of Species Richness in the Park Grass Experiment

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ABSTRACT: The Park Grass Experiment at Rothamsted in southeast England was started in 1856, making it the longest-running experiment in plant ecology anywhere in the world. Experimental inputs include a range of fertilizers (nitrogen, phosphorus, potassium, and organic manures) applied annually, with lime applied occasionally, and these have led to an increase in biomass and, where nitrogen was applied in the form of ammonium sulfate, to substantial decreases in soil pH. The number of species per plot varies from three to 44 per 200 m^2, affording a unique opportunity to study the determinants of plant species richness and to estimate the effect sizes attributable to different factors. The response of species richness to biomass depends on the amount and type of nitrogen applied; richness declined monotonically with increasing biomass on plots receiving no nitrogen or receiving nitrogen in the form of sodium nitrate, but there was no relationship between species richness and biomass on plots acidified by ammonium sulfate application. The response to lime also depended on the type of nitrogen applied; there was no relationship between lime treatment and species richness, except in plots receiving nitrogen in the form of ammonium sulfate, where species richness increased sharply with increasing soil pH. The addition of phosphorus reduced species richness, and application of potassium along with phosphorus reduced species richness further, but the biggest negative effects were when nitrogen and phosphorus were applied together. The analysis demonstrates how multiple factors contribute to the observed diversity patterns and how environmental regulation of species pools can operate at the same spatial and temporal scale as biomass effects.

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The question as to why some areas support more species than others has fascinated naturalists for centuries (Huston 1994; Rosenzweig 1995). Recently, a great deal of effort has been dedicated to discovering the mechanisms that prevent complete dominance by the superior competitor and allow coexistence of less competitive species. At small scales, it is likely that plant-plant (Goldberg and Estabrook 1998), plant-microbe (van der Heijden et al. 1998; Zak et al. 2003), and plant-enemy (Pacala and Crawley 1992) interactions play a central role. There may also be an expectation that local species richness will be higher where the regional pool of species is greater (Collins et al. 2002) or where the disturbance regime is of intermediate severity (Grime 1973).

A persistent difficulty in studies of patterns in species richness is the paradox that species richness increases with productivity in large-scale observational studies but declines when productivity is increased in plot-scale experiments (e.g., by addition of fertilizers; Silvertown 1980; Tilman 1993). At geographic scales, there is a wealth of data showing a positive correlation between primary productivity and species richness (e.g., the latitudinal gradient of species richness; Huston 1994; Rosenzweig 1995). In contrast, experimental increases in productivity via nitrogen (N) addition generally led to decreased species richness (Gough et al. 2000*a*).

Several recent studies report a hump in the species richness–biomass relationship (Pollock et al. 1998; Mittelbach et al. 2001; Venterink et al. 2001; Allcock and Hik 2003), and while most studies are restricted to aboveground biomass, the humped relationship held when belowground biomass was included (Liira and Zobel 2000). On a regional scale in central Europe, there was a hump-shaped relationship between soil nutrient supply and plant species richness within a given biome, but the location of the peak in species richness occurred in different places in different communities; for grasslands and wetlands, the peak was

on nutrient-poor soils, but for forests, the peak was on nutrient-rich soils (Cornwell and Grubb 2003).

Specific nutrients can have characteristic impacts on plant species richness. For example, high plant species richness is typically associated with low levels of soil phosphorus (P) availability (Venterink et al. 2001, 2003). Janssens and colleagues (1998) show that where there was >5 mg P/100 g of dry soil, no location had >20 species/100 m^2. The highest numbers of species were always found below the optimum P content (5–8 mg P/100 g) for the nutrition of most plants. For potassium (K), however, the largest number of species was found at 20 mg/100 g exchangeable K, which is optimum for most plants. Thus, P rather than K has the greater effect on species richness (Janssens et al. 1998).

The size of the total species pool may be the most important determinant of local species richness. In one study, for instance, the positive effect of the species pool was about twice as important as the negative effect of biomass (Safford et al. 2001). Further, there is growing evidence that variation in nonresource environmental factors like soil pH has the potential to regulate species pools in a way that is uncorrelated with aboveground biomass (Gough et al. 2000*b*; Grace 2001).

The Park Grass Experiment (PGE), begun at Rothamsted in 1856 and still running, affords a unique opportunity to test for the determinants of species richness. In this article, we use new data from 1991 to 2000 to address the question, Why do some plots support 44 species in equilibrium while others support only three? The great advantage of the PGE is that most of the plots have been monitored for sufficiently long that their botanical composition is known to be as close to equilibrium as is reasonable to expect in a fluctuating and changing world. We can certainly be reasonably confident that the transient dynamics initiated by the original application of the experimental treatments have been damped. This putative equilibrium does not mean that plots are static in their botanical composition. On the contrary, of 14 species recorded on acidified plots in a 60-year time-series analysis, eight decreased with time, one (*Agrostis capillaris*) increased, four showed no trend, and one (*Chamerion angustifolium*) showed an outbreak with a peak in 1946. On the other hand, of 43 species recorded on nonacidified plots, six increased, five decreased, 10 showed outbreaks (i.e., increase then decline), and 22 showed no trend (Dodd et al. 1995). Analyses of the earlier data on yields and species composition from PGE are to be found elsewhere (Lawes and Gilbert 1859; Brenchley and Warrington 1958; Williams 1978; Silvertown 1980; Tilman 1982; Jenkinson et al. 1994; Silvertown et al. 1994; Tilman et al. 1994).

The objective of this present study was to determine whether multiple factors contribute to the observed diversity patterns and to assess the extent to which species richness is controlled by fine-scale environmental gradients in the species pool. We achieved this by estimating the effect sizes attributable to each of the experimental inputs and related these to variation in soil pH (as a surrogate for the size of the species pool).

Methods

The Park Grass Experiment was set up by Sir John Lawes in 1856 on ancient grassland close to Rothamsted Manor (grid reference TL 124129) with the object of determining the combination of nutrients that gave the maximum yield of hay. With only minor modifications, the same fertilizer treatments have been applied to each of the plots (app. A, in the online edition of the *American Naturalist*) in each of the succeeding 147 years, making this the longest-running manipulative field experiment in all of ecology (Tilman et al. 1994).

Management of the Plots

Details of current management, and of historical changes in management, are described in appendixes A and B in the online edition of the *American Naturalist*. Briefly, the experiment consists of large plots to which different fertilizers are applied, and the large plots are divided into four different liming treatments: lime applied to achieve a target pH 7 (on the *a* subplots) or target pH 6 (*b* subplots, close to the assumed historical, preexperiment pH levels), plots that are limed only if soil pH drops below 5 (*c* subplots; these are always limed when nitrogen is applied in the form of ammonium sulfate, but only some of the others have been limed since 1965), and unlimed (*d* subplots; see apps. D, E in the online edition of the *American Naturalist*).

Transient Plots

In 1989, the plots receiving 96 kg/ha/year N (plot 9, which got ammonium sulfate, and plot 14, which got sodium nitrate) were split in half, and nitrogen application was stopped on one of the halves (these are the "transient plots," plots 9.1 and 14.1); N application continued as previously on the other half (plots 9.2 and 14.2). By transient plots we mean plots showing transient dynamics in their botanical composition; the plots, of course, are permanent. In 1994, all the subplots on plot 13 were halved, and manuring (farmyard manure [FYM] and fish meal) was stopped on plot 13.1 (adjacent to control plot 12) and continued as before on plot 13.2. The original experimental design did not give a complete factorial combination of all treatments, with the result that a number of

potentially interesting treatments were never included (e.g., not until 1996 was there a plot receiving K on its own, when plot 2 was halved to accommodate this extra treatment). Some plots receive similar nutrient inputs, either by design (e.g., plots 7 and 15 both receive only P, K, Na, and Mg) or by circumstance (because lime has never been applied to the less acid *c* subplots [see apps. A, D], the *c* and *d* subplots replicate some of the combinations of fertilizer inputs on plots 14–17; these plots are neighbors, of course, and so are not spatially independent).

Thus, by 1996, the Park Grass Experiment consisted of 97 different combinations of liming and fertilizer inputs (see app. B). For ease of presentation, the *a*, *b*, *c*, and *d* subplots will from now on be referred to as "plots." A map of Park Grass that shows the plot layout is in appendix B, and an aggregate species list for the 97 plots is in appendix C (both in the online edition of the *American Naturalist*). Careful management of the experiment over its entire duration has ensured that the plot boundaries have remained remarkably sharp, whether estimated by species change or by soil parameters (Kunin 1998), indicating that lateral movement of mineral nutrients is negligible in this system.

Herbage Sampling, 1991–2000

From 1991 to 2000, six randomly located quadrats measuring 50 cm × 25 cm were located within each of the plots in early June, immediately before harvesting the first hay crop. The herbage was cut with scissors to ground level and plant material taken back to the laboratory where it was sorted to species. Samples were oven-dried at 80°C for 24 h, after which dry mass was determined for each species. The individual quadrats provide 60 small-scale (0.125 m^2) species richness estimates (D_1) per plot (10 years and six replicates per year). In any given year, the six quadrats in aggregate provide a single species richness estimate (D_2) at 0.75 m^2 for each plot. To estimate whole-plot species density (D_3), each plot was visited monthly from April to November each year, and a composite list of species was compiled. Individual plots vary somewhat in size; the smallest is ~0.013 ha, and the largest is ~0.05 ha. We have no evidence that there were any local extinctions or species invasions during the 10-year period of this study, so we assume that whole-plot species richness was constant.

Statistical Analysis

Counts of species richness were analyzed in a variety of ways (e.g., as generalized linear models using Poisson errors and a log link, corrected for overdispersion as necessary, or as linear models following a variety of transformations of the counts). The different approaches were compared on the basis of model checks (e.g., constancy of variance and normality of errors) using diagnostic plots (Crawley 2002). It turned out that one of the simplest methods was best, so all statistical analyses reported in this article were based on linear modeling with square root transformation of species counts as the response variable. Species richness was measured at three spatial scales, but in no case was the qualitative structure of the minimal adequate model materially affected by the choice of scale at which to measure species richness (although there were small changes in the *P* values). For simplicity, therefore, all of the analyses reported here refer to square root transformed species richness measured at the whole-plot scale ($[D_3]^{1/2}$).

The Park Grass Experiment would not pass muster as an experimental design today. There is no randomization, replication is uneven, treatment combinations are missing, and the lime treatments are confounded with spatial location. Of course, the experiment was designed before modern statistical ideas about replication and randomization had been developed following the pioneering studies of R. A. Fisher at Rothamsted. The size of the plots (>100 m^2) goes some way to compensate for the lack of replication, particularly because the meadow was reasonably uniform before the experiment began (Lawes and Gilbert 1859). The result of these design features is that the data are unbalanced, with the result that sums of squares cannot be unequivocally attributed to all of the important explanatory variables. The sequence in which terms are fitted to or deleted from the model influences the explanatory power attributed to them (order matters). Because of this, we have adopted a protocol for model simplification in which a maximal model (including interaction terms and quadratic terms for continuous explanatory variables) was fitted first, then model simplification involved deletion of variables and reduction of factor levels (Crawley 2002). The explanatory variables are the experimental treatments: categorical variables with two levels in the case of P and K (applied or not); three levels for the type of nitrogen fertilizer (none, ammonium sulfate, or sodium nitrate); four levels for liming; two levels for the transients (equilibrium or transient; the nutrient inputs for the transient plots are scored as their current [i.e., post 1989] reduced inputs without N); two levels for organics (organics applied or not); and one continuous explanatory variable (application rate of N), with two covariates: total first-cut biomass and soil pH.

Results

Species richness was greatest ($D_3 > 40$) on plots that had no extra experimental nutrient inputs. It was lowest

($D_3 < 5$) on plots where the soil was strongly acidified by the long-term input of ammonium sulfate supplying 144 kg N ha^{-1} $year^{-1}$.

Effects of Total Biomass on Species Richness

There was a significant negative correlation between first-cut biomass and species richness (fig. 1*A*; $r = -0.227$, $P = .0253$) for all 97 plots; the scatter is great, and the explanatory power of the relationship is low ($r^2 = 0.052$). There was no hint of a hump in the relationship.

Effects of Soil pH on Species Richness

The pH of each plot in 1995–1998 is given in appendix E. There was a significant positive correlation between species richness and soil pH ($r = 0.568$, df $= 95$), but the relationship is evidently nonlinear (fig. 1*B*). Species richness declines rapidly as pH falls below 4.5, and the lowest species richness ($D_3 = 3$) was found on plot 11.1*d* where pH = 3.6.

Effects of Biomass and pH Together on Species Richness

The effect of biomass on species richness depends on soil pH, and the strength of the relationship varies with soil pH (fig. 2). The negative correlation between biomass (t/ha dry matter from the first cut) and species richness is not evident on the most acidified plots (fig. 2, *lower left panel*) but is pronounced above soil pH 6.2. An initial model containing biomass and pH as two continuous explanatory variables, each with quadratic terms and an interaction term (six parameters), simplified to a four-parameter model with terms for pH ($t = 7.6$, $P < .0001$) and biomass ($t = -5.3$, $P < .0001$) and a quadratic term for pH ($t = -4.2$, $P < .0001$). There was no interaction between the two variables ($P = .059$) and no quadratic term for biomass ($P = .902$). The descriptive power of this very simple model was moderate (multiple $r^2 = 0.64$).

Effects of Experimental Nutrient Inputs

Figure 3 shows the effect of nutrient treatments on mean species richness for the *b* subplots of the liming treatments (to minimize confounding pH effects). Species richness declines from the control plots (*none*, in fig. 3) through plots receiving P alone, sodium nitrate (N*) or ammonium sulfate (N) on their own, N and K together (the minus-P plot), FYM, and P together with K. The largest reductions in species richness are associated with adding N and P together, and maximum depression of species richness occurs when N is applied as ammonium sulfate. Only N

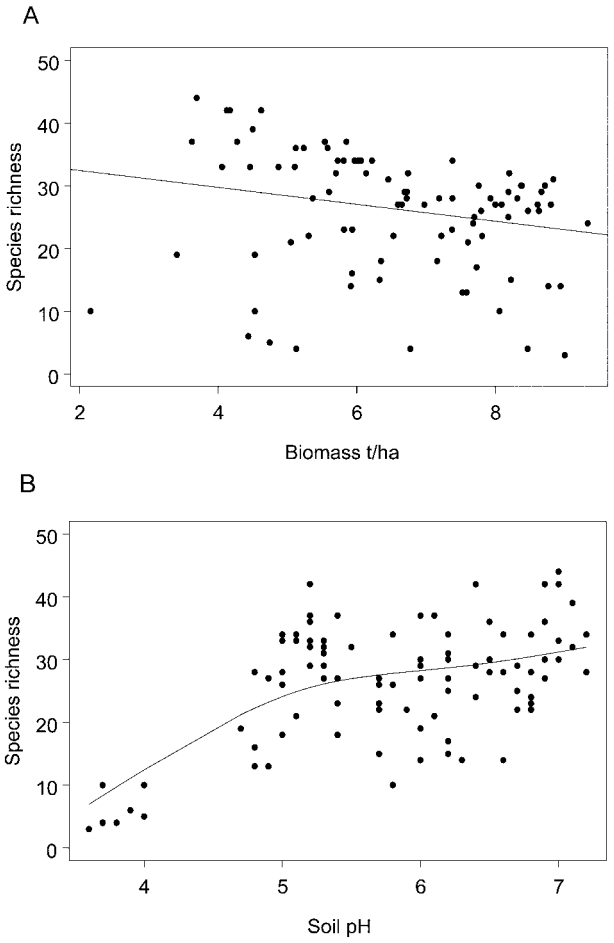

Figure 1: Correlates of whole-plot species richness, D_3. *A*, Species richness declines with increasing biomass, as measured by long-term mean hay yield from the first cut in June (t ha^{-1} dry matter). *B*, Species increases nonlinearly with soil pH. The fitted line is a nonparametric smoother (cubic B-spline smooth, with df = 3.5). The nonlinearity is significant as assessed by the addition of a quadratic term for soil pH to a linear model ($t = -4.166$, df $= 94$, $P < .001$). Each point represents one of the 97 plots.

($P < .00001$) and P ($P < .00001$) had significant main effects on species richness; K ($P = .303$) had no significant effect (table 1). There was no significant interaction between N and P application ($P = .1367$); the effect of adding N and P together was additive, and it was responsible for the greatest reduction in plant species richness attributable to nutrients (in contrast to interactions involving soil pH). On plots receiving N in the form of sodium nitrate, adding P reduced mean species richness from 33.5 to 24.9, whereas on the plots receiving N in the form of acidifying ammonium sulfate, adding P reduced species richness from 25.2 to 15.2. On plots without N, when K was added together with P (plots 7 and 15), mean species

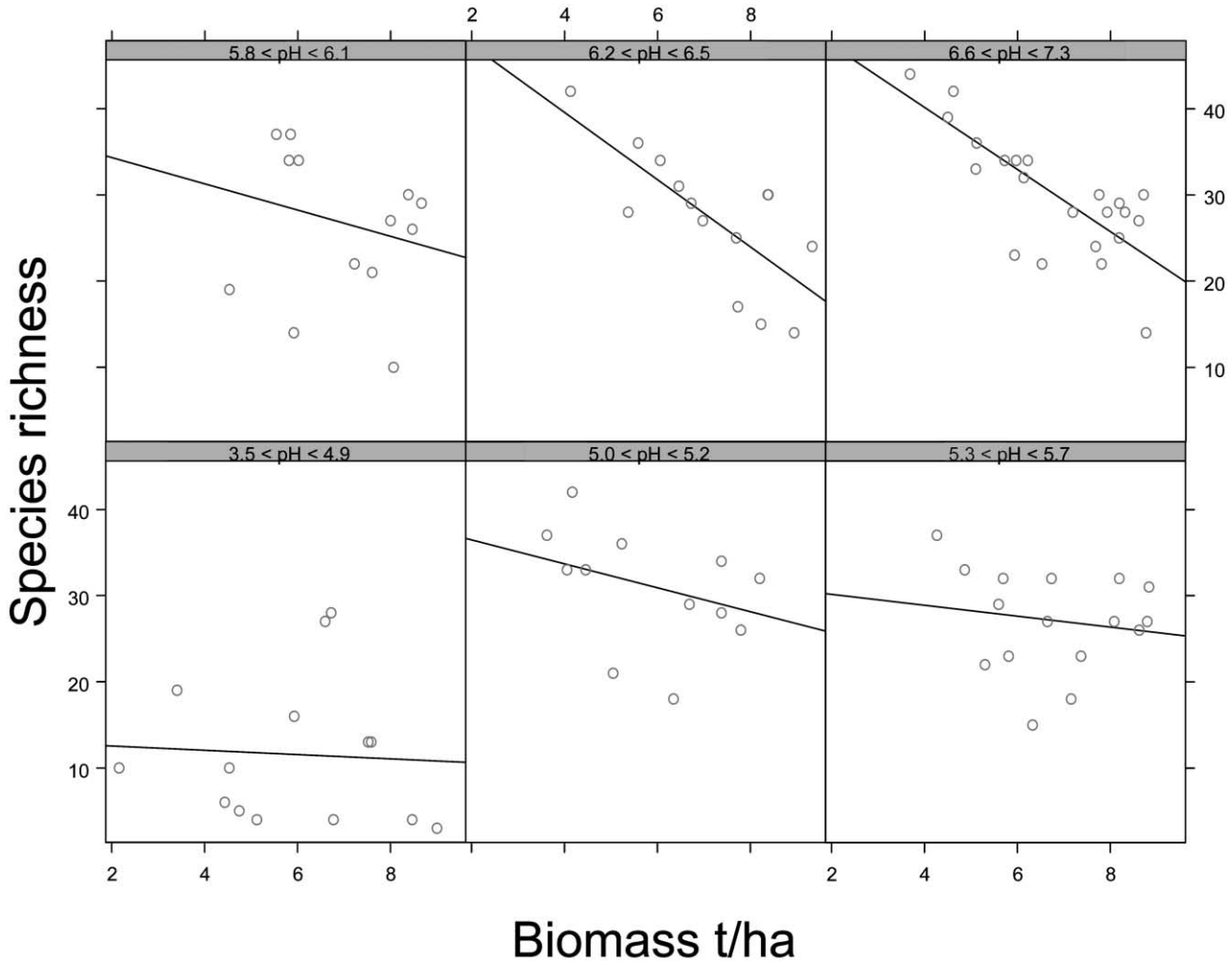

Figure 2: Relationship between whole-plot species richness D_3 and biomass (t ha^{-1}) at six levels of soil pH. The panels are ordered with increasing pH from lower left (pH < 4.9) to upper right (pH > 6.6). The relationship between biomass and species richness becomes progressively steeper as soil pH increases, except in the range 5.3 < pH < 5.7. Lines show separate linear regressions for each panel of pH range.

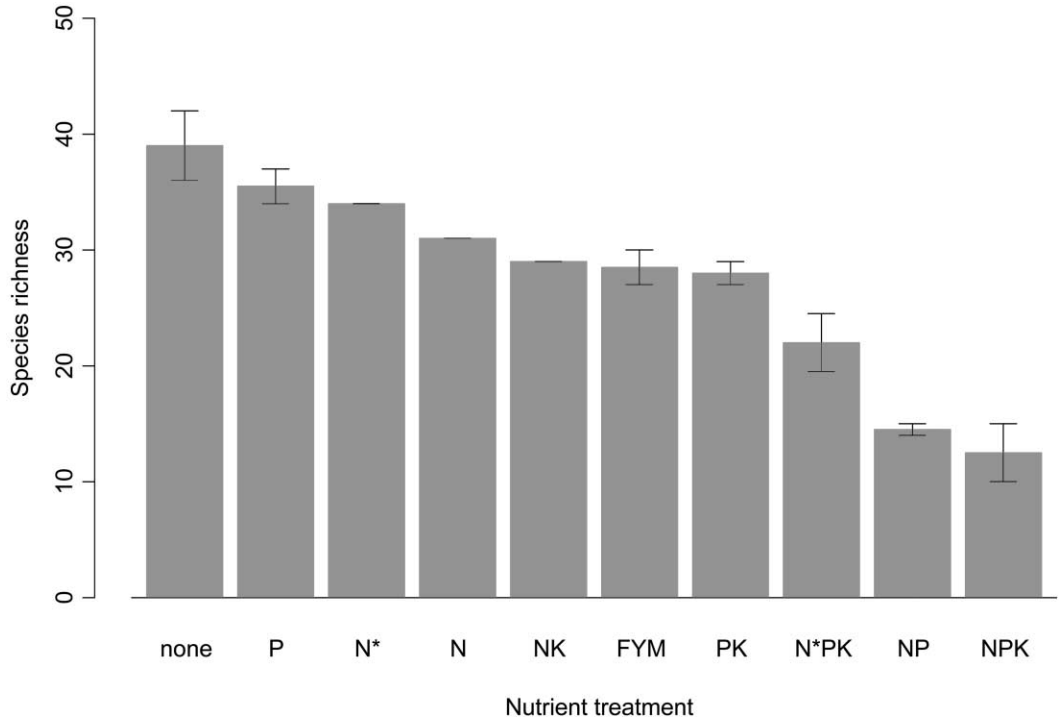

Figure 3: Fertilizer inputs and mean whole-plot species richness (D_3) for the "b" subplots (target pH 6) thus eliminating potentially confounding effects of pH: *none* = plots with no fertilizer inputs, *P* = phosphorus alone, *N** = nitrogen applied on its own as sodium nitrate, *N* = nitrogen applied on its own as ammonium sulfate, *NK* = nitrogen as ammonium sulfate plus potassium (the "minus-P" treatment), *FYM* = farmyard manure, *PK* = phosphorus and potassium applied without nitrogen (the "minus-N" treatment), *N*PK* = all three major nutrients applied together, with N applied as sodium nitrate, *NP* = ammonium sulfate N plus phosphorus (the "minus-K" treatment), *NPK* = all three major nutrients with N applied as ammonium sulfate. The largest reductions in species richness occur when N and P are applied together, and the reduction is greatest when N is applied as ammonium sulfate. Error bars show 1 SE except where there is no replication (there is only one plot receiving nitrogen alone [plot 1 for ammonium sulfate *N*, and plot 17 for sodium nitrate *N**] and only one plot receiving N and K but no P [plot 18.1]). The other SEs are based on two replicates, except *FYM*, where $n = 3$. For significance tests, see table 1.

richness declined from 32.9 to 27.5 compared with plots receiving P alone (plots 4.1 and 8); this decline in species richness was associated with an increase in mean first-cut biomass from 5.90 to 7.76 t ha^{-1} dry matter.

Effects of Nitrogen Fertilizer Type on Species Richness

The relationship between biomass and species richness is most pronounced on the plots that do not receive N (fig. 4*a*), weak on the plots receiving N as sodium nitrate (fig. 4*b*), but nonexistent on the plots receiving N as ammonium sulfate (when soil pH effects are ignored; cf. figs. 4*c*, 2). The overall negative impact of N on species richness is shown by the fact that only 19 of the 54 plots receiving N have more than 26 species, which was the minimum value from a plot where N had not been applied. The effect of nitrogen fertilizer type on mean species richness is clear: no N fertilizer, $\overline{D}_3 = 33.1$; N applied as sodium nitrate, $\overline{D}_3 = 26.7$; N applied as ammonium sulfate, $\overline{D}_3 = 17.8$ (species richnesses averaged over all lime treatments).

Effects of Nitrogen Application Rate on Species Richness

There was a roughly linear decline in mean species richness with N application rate for both types of N (fig. 5). The reduction in species richness per kilogram of added N was significantly greater for N applied in the form of ammonium sulfate than for sodium nitrate ($P = .0221$).

Effects of Experimental Addition of Lime

There was no significant effect of lime addition on mean species richness for plots receiving no nitrogen (fig. 6*a*; $F = 0.44$, df $= 3, 39$, $P = .728$) or on plots receiving N in the form of sodium nitrate (fig. 6*b*; $F = 0.27$, df $= 3, 15$, $P = .845$). For plots receiving N in the form of ammonium sulfate, however, there was a highly significant

Table 1: Minimal adequate model for species richness at the whole-plot scale ($[D_3]^{1/2}$)

Parameter	Coefficient	SE	*t* value	Pr (>|*t*|)
Intercept	3.6659	.3037	12.0704	.0000
pH	.2345	.0542	4.3300	.0000
Lime	−.2358	.0543	−4.3409	.0000
P	.3371	.0371	9.0863	.0000
N amount	−.0098	.0012	−7.9592	.0000
N type 1	−.5404	.0714	−7.5656	.0000
N type 2	.1977	.0369	5.3569	.0000
Organics	−.2412	.0589	−4.0958	.0001
Transient	−.2607	.0527	−4.9429	.0000
Lime N type 1	−.4644	.0463	−10.0334	.0000
Lime N type 2	.1292	.0346	3.7377	.0003

Note: Minimal adequate model for species richness at the whole-plot scale ($[D_3]^{1/2}$) as a function of experimental treatments (categorical variables for the type of nitrogen applied [three levels], phosphorus [two levels], organic manures [three levels], transients [two levels], liming treatment [four levels initially], and a continuous variable for the amount of nitrogen applied) and continuous covariates (biomass and soil pH). Explanatory variables excluded during model simplification included biomass ($P = .188$) and potassium ($P = .303$), along with quadratic terms for biomass ($P = .809$) and pH ($P = .176$) and an interaction term between biomass and pH ($P = .073$). Lime treatment was simplified from a four-level factor to a two-level factor (limed or not, $P = .0001$), and organics was simplified to a two-level factor (organic manures applied or not, $P = .0116$). There was a highly significant interaction between nitrogen type and liming ($P < .00001$). This model explains 92% of the variation in species richness using 11 parameters with df = 86 for residual variation. Residual SE = 0.3204, df = 86, $R^2 = 0.9226$, $F = 102.5$, df = 10, 86, $P = 0$.

impact of lime application on species richness (fig. 6*c*). Model simplification indicates that this can be attributed to the single-degree-of-freedom contrast between the unlimed *d* plots and the others (*a*, *b*, and *c* taken together; $F = 54.54$, df = 1, 33, $P < .00001$).

A Statistical Model with both Categorical and Continuous Explanatory Variables

The minimal adequate model for species richness at the whole-plot scale ($[D_3]^{1/2}$) as a function of experimental treatments (categorical variables for the type of N and P applied, organic manures, liming treatment, and a continuous variable for the amount of N applied) and continuous covariates (biomass and soil pH) explained 92% of the variation in species richness with 11 parameters and df = 86 for residual variation. Effect sizes and the significance levels associated with parameters deleted during model simplification are shown in table 1. There was only one significant interaction: species richness was significantly lower on unlimed plots receiving ammonium sulfate (N type = 1) than on unlimed plots receiving sodium nitrate (N type = 2) because of their different effects on soil pH.

Changes in Species Richness on Transient Plots with Altered Nutrient Inputs

The transient plots on which N input was stopped in 1989 (plot 9 receiving N as ammonium sulfate and plot 14 receiving N as sodium nitrate) make an interesting contrast to the equilibrium plots. Park Grass is unique because we know the likely equilibrium end points for these transient plots with altered inputs (from the long-term P and K [plots 7 and 15] without N). Species richness of the transient plots is compared with initial and likely final conditions in figure 7, which shows fewer species on the equilibrium NPK than on the equilibrium PK plots. Recovery of species richness (and also convergence of species identities) was rapid on the sodium nitrate plots but much slower on the acidified ammonium sulfate plots. Within each N fertilizer type, recovery was more rapid on the limed parts of the plots than on the more acidic parts. The unlimed plot 9.1*d* had accumulated only one extra species in the period 1989–2000, presumably because low pH rather than N supply controls species number, and pH is likely to increase only slowly now that ammonium sulfate is no longer applied. There is no evidence that species richness was higher on any of the transient plots than on equilibrium end points (the PK [P plus K] plots).

Species-Area Relationships

The species-area curves are shown separately for each plot in figure 8. There is very little crossing over (i.e., "tracking" is high), which means that the broad structure of the minimal adequate model for determination of species richness does not depend on the spatial scale chosen for the response variable. The analysis of the slopes (*z* values) of the species-area relationships (on log-log axes) is described in appendix F in the online edition of the *American Naturalist*. The slopes were significantly higher for the first density transition (z_1 from mean species richness at 0.125 m^2 to mean at 0.75 m^2) than for the second (z_2 from mean at 0.75 m^2 to whole-plot scale). Factors affecting differences in slope are described in appendix F.

Discussion

The broad correlates of species richness on Park Grass are well known (Silvertown 1980); species richness declines with increasing biomass and increases with increasing soil pH (fig. 1). Here, we have been able to demonstrate the effect sizes attributable to different experimental treatments and to show that these patterns do not depend on the spatial scale at which species richness is measured. Experimental inputs associated with increases in biomass (N, P, and K) cause essentially linear reductions in species

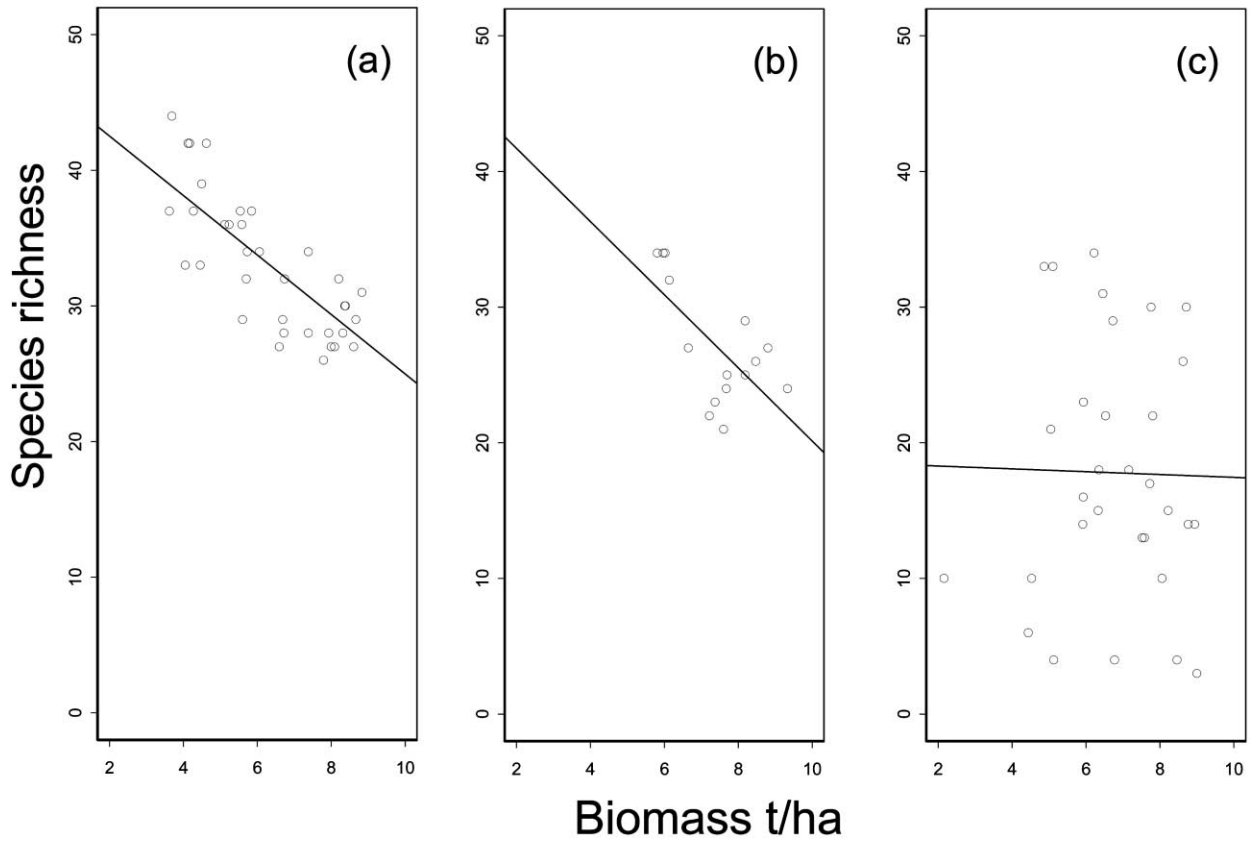

Figure 4: Nitrogen fertilizer type and plant species richness (D_3) as a function of total biomass. *a*, Relationship between species richness and biomass (long-term mean hay yield at the first cut in June; t ha^{-1} dry matter) is linear and well defined on plots receiving no nitrogen fertilizer. *b*, Relationship is less well defined when nitrogen is applied in the form of sodium nitrate. *c*, When soil pH is ignored, there is no correlation between species richness and biomass for plots receiving nitrogen in the form of ammonium sulfate.

richness so long as there is no concomitant reduction in soil pH. Application of N in the form of ammonium sulfate causes reduced soil pH, and this in turn acts to reduce the size of the species pool. Overall, therefore, it is the interaction between biomass and nitrogen type that is the principal determinant of plant species richness: in non-acidified cases, the biomass effect is most important, while at low pH the pool size effect is central (figs. 2, 4). Liming acts to increase species richness on plots fertilized with ammonium sulfate but not in other cases (fig. 6).

The treatment effects are reasonably clear cut. The unfertilized control plots exhibit maximum species richness ($D_3 > 35$) and support species that are simultaneously good competitors for N, P, and K. Individual plants are small, and species coexist in a fine-grained, herb-rich community. Any nutrient addition causes a long term decline in species richness. Sadly, PGE gives us no direct insight into the mechanisms that allow equilibrium coexistence of >30 perennial plant species on these plots.

A simple four-parameter regression model described the patterns shown in figures 1 and 2 (an intercept, a negative slope for biomass, a positive slope for soil pH, and a negative quadratic term for soil pH) and accounted for 64% of the variation in $(D_3)^{1/2}$. Incorporating the experimental treatments (figs. 3, 6) instead of biomass and pH explained 86% of the variation in $(D_3)^{1/2}$, with the interaction between N fertilizer type and lime as the principal explanatory term. This is because the lowest species richness is consistently found on unlimed *d* plots that received N as ammonium sulfate. A combined model with experimental treatments and continuous covariates for biomass and pH explained 92% of the variation in species richness using only 11 parameters with df = 86 for residual variation (table 1). Roughly speaking, the model predicts that adding P loses six species on average; adding N loses about two species for every 50 kg ha^{-1} applied; ammonium N loses three more species than would the same rate of N as sodium nitrate (because of the effect on soil pH); two extra

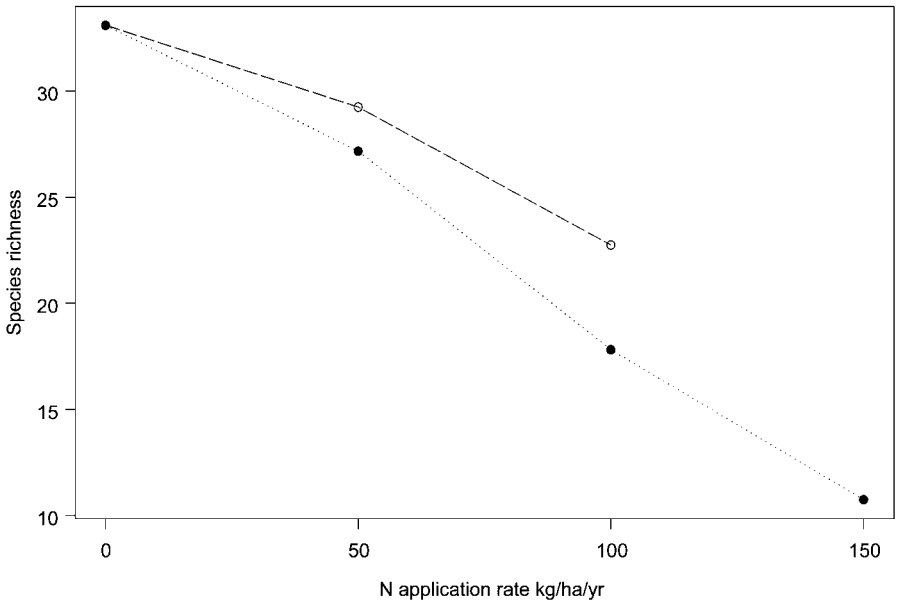

Figure 5: Relationship between mean plant species richness and the amount of nitrogen applied (kg ha^{-1} $year^{-1}$). Species are lost roughly linearly over the range 0–150 kg N ha^{-1} $year^{-1}$, but species are lost less rapidly from plots receiving nitrogen applied in the form of nonacidifying sodium nitrate (*open circles*) than as acidifying ammonium sulfate (*solid circles*). Analysis of covariance using linear regression indicates that the two slopes are significantly different ($P = .0221$).

species are gained for every unit increase in soil pH, while liming the ammonium sulfate plots adds 15 species compared with the unlimed *d* plots; and using organic manures rather than mineral fertilizers adds two species on average. In terms of effect sizes, therefore, the interaction between liming and N fertilizer type is greatest (>15 species), followed by the amount of N applied (~6 species), soil pH (~6 species), and P application (~6 species), with smaller but significant effects for organic fertilizer (positive) and transience (negative).

The equilibrium plots at Park Grass provide no support for the hypothesis of a humped relationship between species richness and productivity or biomass. There was a clear negative relationship between species richness and biomass for all treatments except those where soil pH lay in the range $5.3 < pH < 5.7$, where the relationship was flat (fig. 2). The situation is different on the eight transient plots where N input was stopped after 1989; these show a nonsignificant positive correlation between species richness and biomass. It is plausible that positive correlations between biomass and species richness could be a feature of transient dynamics (e.g., early successional systems or recently established biodiversity experiments) where the sampling effect is more likely to be important (Hector 2001; Pacala and Tilman 2001).

In terms of mechanisms, PGE provides support for both the competitive-exclusion and pool-size hypotheses, although it is not clear how much of the apparent effect of competitive exclusion can be attributed to increased plant size and reduced stem density (Stevens and Carson 1999). Adding N and P in combination leads to increased biomass and causes substantially reduced species richness, with the replacement of short herbs by tall grasses presumably a result of competitive exclusion through shading (fig. 3; see Guo and Berry 1998; Gross et al. 2000; He et al. 2002). The pool size effect is seen most clearly in the context of extreme pH. Few species in the Rothamsted flora, other than the heavy metal "excluders," *Anthoxanthum odoratum* found on plot 1*d* and 4/2*d* and *Holcus lanatus* on plot 11*d*, can tolerate the high levels of aluminium experienced on the very acid plots.

The Park Grass plots are relatively species rich across the range of sampled scales (fig. 8) compared with the more acid grasslands in Silwood Park investigated by Crawley and Harral (2001); this pattern is in line with the generally higher species richness on soils of higher pH (Gough et al. 2000*b*). The scale dependence of the slope of the species-area relationship is different on Park grass than in Silwood Park; on Park grass, the slope of the second transition (mean $z_2 = 0.086$) was much shallower under most experimental treatments than the slope of the first transition (mean $z_1 = 0.288$; app. F), whereas in Silwood the two slopes were similar (0.238 and 0.207, respectively; Crawley and Harral 2001). Obvious differences between

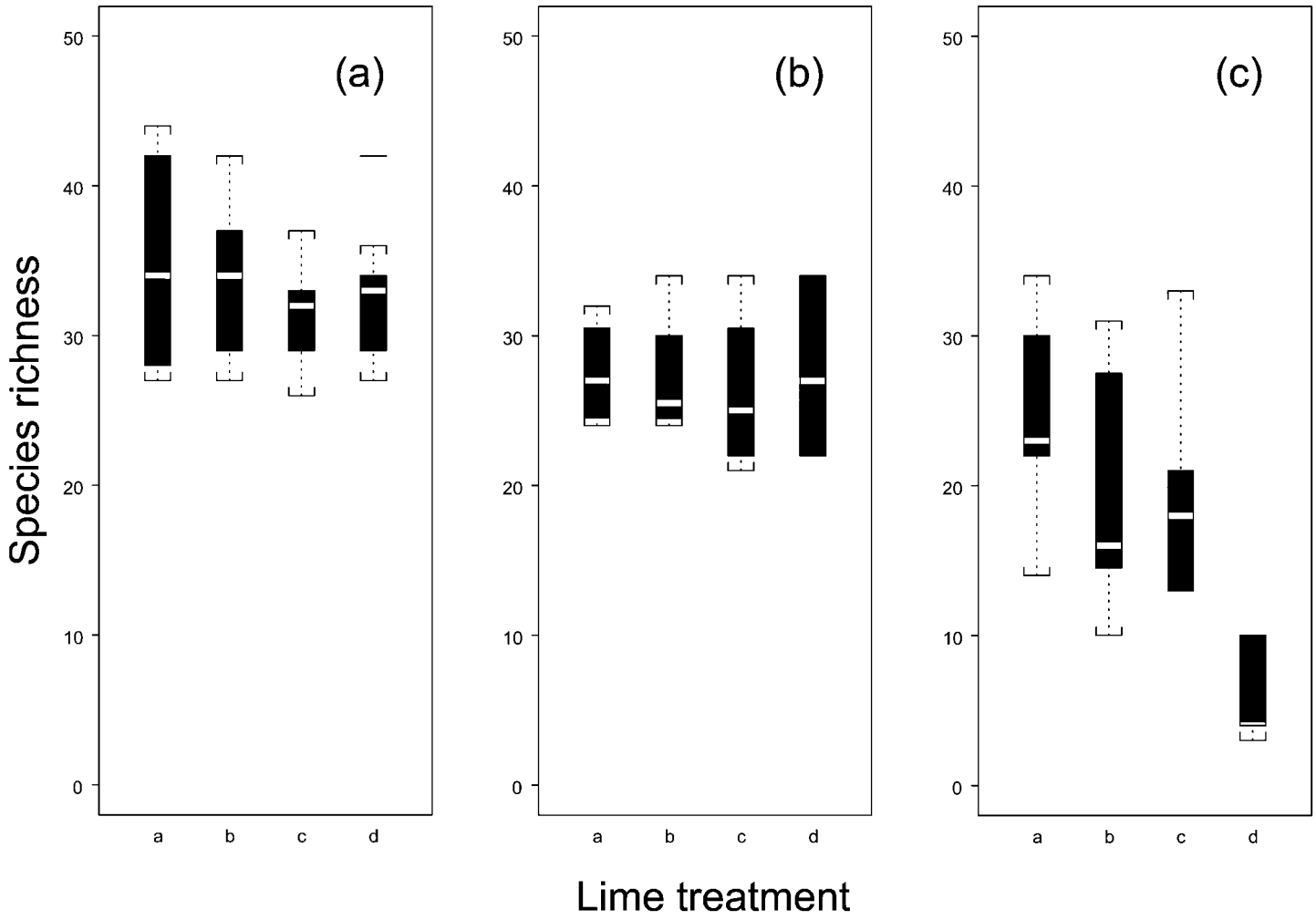

Figure 6: Effects of four levels of liming treatment on median plant species richness (*horizontal white lines*) and interquartile range (*solid bars*). Lime level *a* is limed to a target pH 7, level *b* to a target pH 6, level *c* is limed only if pH falls below a target pH 5, and level *d* represents unlimed plots, varying in pH depending on inputs (the most acidified plots are those receiving N as ammonium sulfate). *a*, There is no significant effect of lime treatment on mean species richness on plots that receive no N fertilizer, but the range of species richness is reduced on unlimed plots. *b*, There is no significant effect of lime treatment on mean species richness on plots that receive N fertilizer in the form of sodium nitrate. *c*, There is a highly significant effect of liming on species richness in plots receiving N in the form of ammonium sulfate. The significance is attributable to the single degree of freedom contrast between the unlimed *d* plots and the others. The effect of nitrogen type on mean species richness is clear: *a*, no N fertilizer, $\overline{D}_3 = 33.1$; *b*, N applied as sodium nitrate, $\overline{D}_3 = 26.7$; *c*, N applied as ammonium sulfate, $\overline{D}_3 = 17.8$.

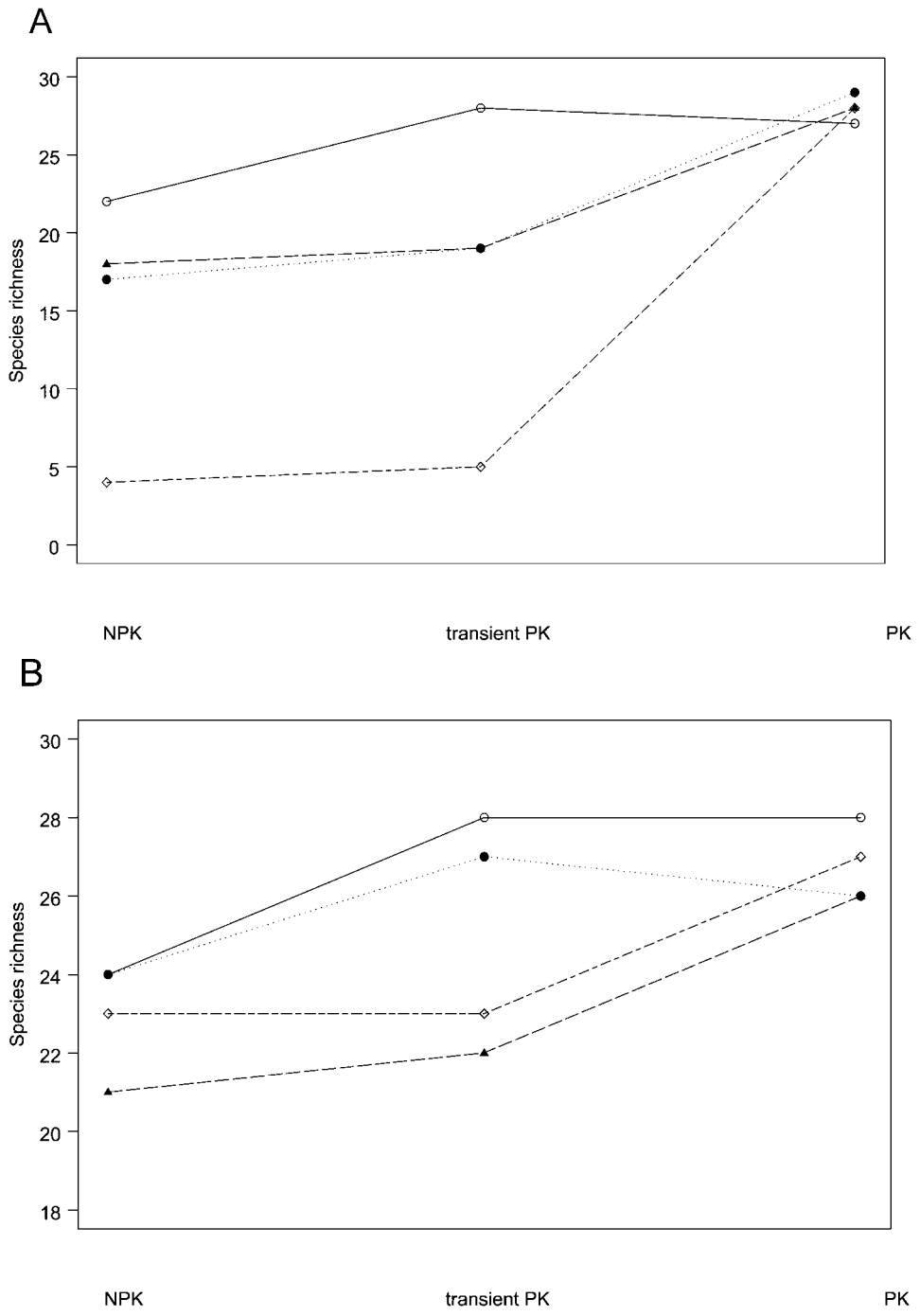

Figure 7: Species richness on the transient plots (9.1 and 14.1) where nitrogen was last applied in 1989. The equilibrium communities receiving NPK (plots 9.2 and 14.2) were the starting points for the transient dynamics, and the equilibrium communities receiving PK are the assumed end points to which the transients are moving (plots 7 and 15). *A*, Ammonium sulfate plots (plot 9.1 = NPK; 9.2 = transient PK) and equilibrium PK (plot 7). *B*, Sodium nitrate plots (plot 14.1 = NPK, 14.2 = transient PK) and equilibrium PK (plot 15). N = nitrogen, P = phosphorus, K = potassium. Symbols show the four liming treatments: *open circles* = *a* plots, *solid circles* = *b* plots, *solid triangles* = *c* plots, *open diamonds* = unlimed *d* plots.

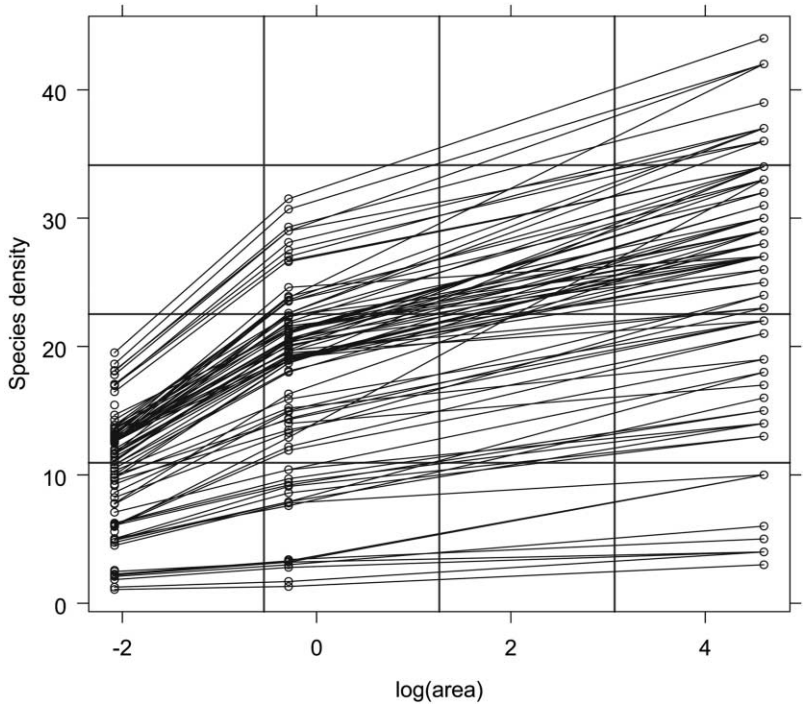

Figure 8: Species-area relationships for each of the nutrient and lime treatments over three spatial scales (0.125-m^2 quadrats, 0.75-m^2 aggregated quadrats, and ~100-m^2 whole plots). Rank species richness is consistent—few of the lines cross over, but the variance in species number increases with increasing spatial scale. On average, the slope is significantly steeper for the first transition (z_1 on log-log axes) than for the second (z_2) as detailed in appendix F in the online edition of the *American Naturalist*. Steep second transitions occur on plots where many rare species are represented by a few isolated individuals. The very flat relationships at extremely low species richness are for the unlimed *d* plots receiving high rates of nitrogen as ammonium sulfate.

the two locations include the fact that Silwood has more acid soils and is heavily grazed by rabbits while Park grass is mown.

The PGE plots make some important points for the management of conservation grasslands. It is often thought that N input is anathema for plant biodiversity, but PGE shows that this is not necessarily so if the N input is nonacidifying and if N addition is not accompanied by other limiting nutrients like P. For instance, the addition of N in the form of sodium nitrate was not nearly as damaging to species richness as the application of acidifying ammonium sulfate. It is noteworthy that the limed plot 17*b*, receiving N^* alone, has one of the highest of all species richnesses ($D_3 = 34$). It could be that the addition of N without P or K will prove to be a valuable technique in grassland restoration projects where the object is to reduce fertility and reinstate species-rich communities (Marrs 1993; Van der Woude et al. 1994; Edwards et al. 2002). The key to the success of this method would be to ensure that as much of the primary production was harvested and carried off site, thereby decreasing soil P levels.

There is no evidence that species richness at the whole-plot scale varied over the 10 years of this study, but there was significant variation in mean species richness at smaller spatial scales from year to year and from replicate to replicate within plots within years (app. F). This variation is likely the result of changes in relative abundance of different species reflecting growth responses in different kinds of years (e.g., wet years or dry years) and differences in mean plant size and growth form across species. Evidently, the year effect was slightly less than the spatial heterogeneity component; this is a response that has not been widely documented but has implications for the temporal heterogeneity model of coexistence (Chesson 1994).

This unique experiment has allowed us to estimate the effect sizes of different inputs on equilibrium and transient species richness. The key insight is that all nutrient additions caused reduced species richness compared with the unfertilized plots. The reductions were greatest when N and P were applied together and soil pH was reduced. The bad news for grassland biodiversity is that eutrophication by N and P pollution is often associated with increased acidity from atmospheric inputs. The analysis of the Park Grass plots has shown how multiple factors contribute to

the observed patterns of plant species richness and has demonstrated that environmental regulation of species pools can operate at the same spatial and temporal scale as biomass effects.

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