

Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the USA

Samuel M. Simkin^{a,1}, Edith B. Allen^b, William D. Bowman^a, Christopher M. Clark^c, Jayne Belnap^d, Matthew L. Brooks^e, Brian S. Cade^f, Scott L. Collins^g, Linda H. Geiser^h, Frank S. Gilliamⁱ, Sarah E. Jovan^j, Linda H. Pardo^k, Bethany K. Schulz^l, Carly J. Stevens^m, Katharine N. Suding^a, Heather L. Throopⁿ, and Donald M. Waller^o

^aUniversity of Colorado, INSTAAR, Boulder, CO 80309; ^bUniversity of California, Riverside, Department of Botany and Plant Sciences and Center for Conservation Biology, Riverside, CA 92521; ^cU.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, D.C. 20460; ^dU.S. Geological Survey, Southwest Biological Science Center, Moab, UT 84532; ^eU.S. Geological Survey, Western Ecological Research Center, Oakhurst, CA 93644; ^fU.S. Geological Survey, Fort Collins Science Center, Fort Collins, CO 80226; ^gUniversity of New Mexico, Department of Biology, Albuquerque, NM 87131; ^hUSDA Forest Service, Pacific Northwest Region Air Resource Management Program, Corvallis, OR 97339; ⁱMarshall University, Department of Biological Sciences, Huntington, WV 25755; ^jUSDA Forest Service, Forest Inventory and Analysis Program, Portland, OR 97339; ^kUSDA Forest Service, Northern Research Station, Burlington, VT 05405; ^lUSDA Forest Service, Forest Inventory and Analysis Program, Anchorage, AK 99501; ^mLancaster University, Lancaster Environment Centre, Lancaster, LA1 4YQ, United Kingdom; ⁿArizona State University, School of Earth and Space Exploration & School of Life Sciences; and ^oUniversity of Wisconsin, Department of Botany, Madison, WI 53706

Submitted to Proceedings of the National Academy of Sciences of the United States of America

Atmospheric nitrogen (N) deposition has been shown to decrease plant species richness along regional deposition gradients in Europe and in experimental manipulations. However, the general response of species richness to N deposition across different vegetation types, soil conditions, and climates remains largely unknown even though responses may be contingent on these environmental factors. We assessed the effect of N deposition on herbaceous richness for 15,136 forest, woodland, shrubland, and grassland sites across the continental U.S., to address how edaphic and climatic conditions altered vulnerability to this stressor. In our dataset, with N deposition ranging from 1-24 kg N ha⁻¹ yr⁻¹, we found a unimodal relationship; richness increased at low deposition levels and decreased above 8.7 and 13.4 kg N ha⁻¹ yr⁻¹ in open and closed-canopy vegetation, respectively. N deposition exceeded critical loads for loss of plant species richness in 24% of 15,136 sites examined nationwide. There were negative relationships between species richness and N deposition in 36% of 44 community gradients. Vulnerability to N deposition was consistently higher in more acidic soils, while the moderating roles of temperature and precipitation varied across scales. We demonstrate here that negative relationships between N deposition and species richness are common, albeit not universal, and that fine-scale processes can moderate vegetation responses to N deposition. Our results highlight the importance of contingent factors when estimating ecosystem vulnerability to N deposition, and suggest that N deposition is affecting species richness in forested and non-forest systems across much of the continental U.S.

nitrogen deposition | plant species richness | diversity | soil pH | climate

Global emissions of reactive nitrogen (N) to the atmosphere and subsequent deposition into terrestrial ecosystems have tripled in the last century (1). This N deposition has been identified as a threat to plant diversity (2-4), and plant diversity is linked to ecosystem stability (5), productivity (6), and other ecosystem services (7). Elevated nitrogen inputs have been shown to cause decreases in species richness over time in small plot experiments (8-10) and in regional gradient studies in Europe (11, 12). While these and other studies have led to some generalizations about the impacts of N deposition on plant diversity, most of these studies have focused on grassland ecosystems, and/or in the U.S. have been fine-scale field experiments where N is added experimentally as fertilizer. Thus, translation of these findings to non-grassland systems or to large regions of the U.S. may not be appropriate. Unlike grasslands where elevated N has often led to light-limitations and subsequent competitive exclusion (13), plant

growth in the herbaceous layers of forest understories is typically primarily light-limited (14) regardless of the extent of N inputs. Moreover, soil chemistry can be heterogeneous, influencing the potential of soil acidification by nitrogen deposition (15). In most arid ecosystems, moisture may be more important than nutrients in controlling plant growth during the growing season (16, 17). Finally, the level of N input at which diversity is first impacted (18) is often unknown for many regions since most studies use a fairly coarse experimental approach to estimate thresholds of response or have been conducted where there have already been high inputs of N for decades (e.g. northern Europe). To address these critical gaps in our knowledge of continental-scale relationships between N deposition and plant diversity, we used data from herbaceous ground-layer communities within 15,136 forest, woodland, shrubland, and grassland sites spanning N deposition gradients across the continental USA. More specifically, we assessed how co-varying climate and edaphic factors affected ecosystem vulnerability to N deposition.

Nitrogen inputs can either increase or decrease diversity, contingent on a host of associated ecosystem factors. Biodiversity can be reduced through several general mechanisms including but not limited to (4): 1) release from N limitation that leads

Significance

Human activities have elevated nitrogen (N) deposition, and there is evidence that deposition impacts species diversity, but spatially extensive and context-specific estimates of N loads at which species losses begin remain elusive. Across a wide range of climates, soil conditions, and vegetation types in the USA, we found that 24% of >15,000 sites were susceptible to N deposition induced species loss. Grasslands, shrublands, and woodlands were susceptible to species losses at lower loads of N deposition than forests, and susceptibility to species losses increased in acidic soils. These findings are pertinent to the protection of biodiversity and human welfare and should be considered when establishing air quality standards.

Reserved for Publication Footnotes

Table 1. Parameter coefficients for species richness from median quantile regressions.

Name	Open canopy (+/- 1 SE)	Closed canopy (+/- 1 SE)
Intercept	14.9 (3.42)*	13.6 (2.55)*
N	4.69 (0.60)*	0.449 (0.33) ⁿ
N*N	-0.494 (0.02)*	-0.125 (0.01)*
pH	-2.17 (0.46)*	-1.49 (0.37)*
precip	-0.011 (0.002)*	-0.003 (0.001)*
temp	-0.059 (0.18) ⁿ	-0.321 (0.04)*
N:pH	0.475 (0.07)*	0.543 (0.04)*
N:precip	0.002 (0.001)*	NA
N:temp	-0.073 (0.03)*	NA

Regressions represent herbaceous plant species richness response to N deposition ($\text{kg ha}^{-1} \text{ yr}^{-1}$; quadratic), soil pH, total annual precipitation (mm), average annual temperature (deg. C), and interactions of N (deposition) with pH, precipitation, and temperature. Sample size is 11,819 sites for closed canopy (deciduous forest, evergreen forest, and mixed forest) and 3,317 sites for open canopy (grassland, shrubland, and woodland). Level of significance is indicated as n ($P \geq 0.05$), + ($P < 0.05$), or * ($P < 0.001$).

to increased aboveground production, reduced light availability, and ultimately competitive exclusion (13, 19) and 2) soil acidification and associated cation depletion and imbalances that lead to recruitment inhibitions (20, 21). The importance of N limitation likely declines in arid areas that are more moisture limited, or in warm wet areas favoring high net N mineralization that reduce the importance of external N inputs. In such cases, N may be less limiting to plant growth and therefore communities are less responsive to additional N deposition (2). Conversely, enrichment may increase biodiversity in extremely N-poor environments where release from N limitation does not result in competitive exclusion (22, 23) or where soils have a high pH resistant to soil acidification (11, 24).

Because N enrichment can affect plant diversity through multiple pathways and environmental contingencies, we investigated whether N deposition is a widespread threat to plant species diversity or whether some vegetation types or environments are more vulnerable than others. We compiled herbaceous plant species composition data from existing datasets (Table S1) that included 15,136 sites and 3,852 herbaceous species from across the continental USA. At each site, we calculated species richness, the total number of unique species per plot, a commonly used metric of diversity (25). We then extracted geospatial estimates (Table S2) of N deposition, annual precipitation, mean annual temperature, and soil pH for each site. As in several previous studies in Europe (11, 12, 26), we utilized a correlative approach which cannot show direct causality but can nevertheless provide insight into the mechanisms involved in, and communities most susceptible to, loss of diversity as a result of N deposition. First we analyzed relationships between plant species richness and N deposition involving interactions with precipitation, temperature, and soil pH within two broadly defined vegetation types (closed canopy forest vs. open canopy grasslands, shrublands, and woodlands). We then examined the same set of predictors within gradients defined by unique combinations of specific vegetation communities and source datasets that spanned an adequate range of N deposition (Methods, Table S3).

Results and Discussion

National-Scale N Deposition Critical Loads and Exceedances Analyses. At a national scale, separating sites into open canopy (grassland, shrubland, woodland) versus closed canopy (forested) vegetation types, we found that herbaceous plant species richness was best explained by N deposition (R^1 coefficient of determination = 0.10 and 0.05 for open and closed vegetation, respectively),

followed by soil pH ($R^1 = 0.02$ and 0.04 for open and closed vegetation, respectively), temperature ($R^1 = 0.04$ and 0.01 for open and closed vegetation, respectively), and precipitation ($R^1 = 0.02$ and 0.004 for open and closed vegetation, respectively). Regression analyses incorporating N deposition interaction effects with other predictors (Table 1) showed strong hump-shaped relationships between herbaceous plant species richness and N deposition in open canopy vegetation (Fig. 1a, Fig. S1a-S1b). In open-canopy vegetation, richness declined at lower N deposition levels in more acidic soils – declining with N deposition above $6.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ at a soil pH of 4.5, and declining with N deposition above $8.8 \text{ kg ha}^{-1} \text{ yr}^{-1}$ at a soil pH of 7 (Fig. 1a). In closed-canopy conditions, the interaction of N deposition with soil pH was even stronger: at a soil pH of 4.5 richness began declining when N deposition exceeded $11.6 \text{ kg ha}^{-1} \text{ yr}^{-1}$, while at the highest pH (8.2) there was no evidence of a decline (Fig. 1b). In closed-canopy communities, there was no significant interaction of temperature (Fig. S1c) or precipitation (Fig. S1d) with N deposition in most quantiles.

Our results demonstrate for the first time across a wide spatial domain that multiple mechanisms may operate to influence the response of plant species richness to N deposition. A decline in species richness with N deposition at low soil pH in both open and closed canopy systems is consistent with the soil acidification mechanism of species loss (20). At higher soil pH, the patterns found in the two systems diverged. Increased species richness with N deposition in the shaded forest understory is consistent with release from the soil acidification mechanism combined with a limited potential for competitive exclusion through shading - because most understory forest species are already well adapted to shady conditions. In open canopy systems, some species are not well adapted to shady conditions, meaning that even though release from soil acidification had occurred at higher pH, competitive exclusion from light limitation may still have been a potential factor affecting plant richness (13).

Critical loads of N deposition based on changes in herbaceous plant species richness are defined as the point at which species losses begin to occur (18) and are calculated here by taking the partial derivative with respect to nitrogen of the surfaces in Figure 1 (and Table 1) and solving for N (see Methods). Critical loads were generally much lower in open grasslands, shrublands, and woodlands than in closed-canopy forests (Table 2, Fig. 2). Critical load estimates were contingent on soil pH (and in open vegetation on climate as well), but parameter uncertainty in the critical loads estimates was relatively modest (Table 2, Fig. S3, Fig. S4). When we subtracted N deposition critical loads estimates from N deposition values we found that 5% of sites had exceedances of $3\text{-}8 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and 19% had exceedances of up to $3 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Fig. S5). For alternate exceedance calculations, a benefit of doubt approach (using upper limit of 95% CI of the critical load) yields a maximum exceedance of $8.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and 18% percent of sites having positive exceedances, while a precautionary approach (using lower limit of 95% CI of the critical load) yields a maximum of $9.6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and 29% percent of sites with positive exceedances. If methods change N deposition estimates, then critical loads would also increase or decrease by that same percentage.

When we applied national-scale critical loads equations (Table 2) to specific level 1 ecoregions we were able to refine (Table S4) previous estimated critical loads (18) as a consequence of utilizing many more data than were previously available across a wider range of environmental conditions. We emphasize that all critical loads of N deposition presented here are for total herbaceous plant species richness from the national analysis, and that critical loads may be lower for specific species (23), functional groups (4), or ecoregions.

273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340

341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408

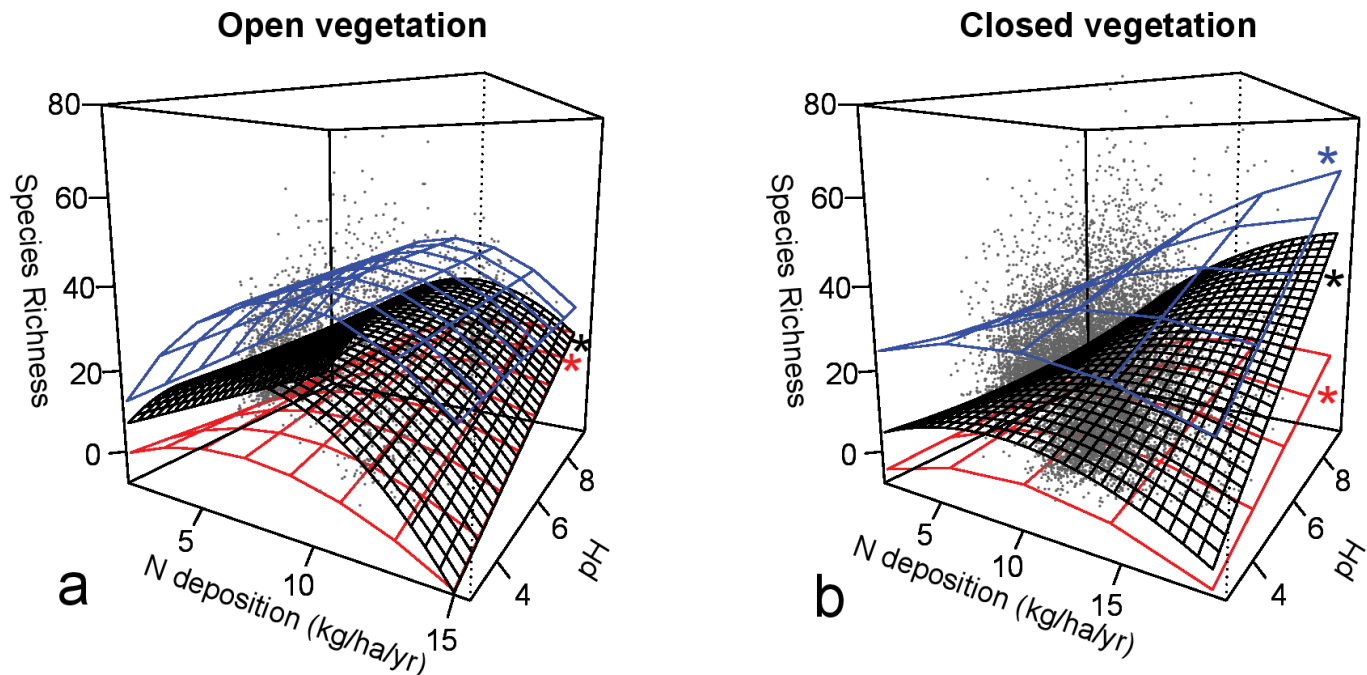


Fig. 1. Herbaceous plant species richness relationships with N deposition. Raw data points ($n = 15,136$ sites) are gray. Surface plots represent 0.1 (red), 0.5 (median; black), and 0.9 (blue) quantile regression models (median parameters in Table 1) fitted to 3,317 open sites (combined grassland, shrubland, and woodland) (a) and 11,819 closed canopy sites (combined deciduous, coniferous, and mixed forests) (b), as influenced by soil pH. Asterisks indicate significant interactions ($P < 0.05$).

Table 2. Critical loads (CL) of N deposition for herbaceous plant species richness.

Vegetation	CL expression (partial derivative of species richness equation)	CL ($\text{kg N ha}^{-1} \text{ yr}^{-1}$)			CL error ^c
		mean ^a	range ^a	Range of 95% CI ^b	
Open canopy vegetation	$[4.690 + (0.475 * (\text{soil pH})) + (0.0018 * (\text{mm of precip.})) + (-0.073 * (\text{temp. } (^{\circ}\text{C})))] / (-2 * -0.494)$	8.7	7.4-10.3	6.4-11.3	-4.5%, 4.8%
Closed canopy vegetation	$[0.449 + (0.543 * (\text{soil pH}))] / (-2 * -0.125)$	13.4	7.9-19.6	6.8-22.2	-6.2%, 7.7%

The critical load (CL) expression is derived using the partial derivative with respect to nitrogen of the species richness equation in Table 1, and then evaluated locally with site-specific soil pH, precipitation, and temperature values. ^aMean and range of CLs across sites, reflecting variation in soil pH, precipitation, and temperature variables across sites but not uncertainty in coefficient estimates. ^bRange of CL 95% confidence interval endpoints across sites (see Fig. S3), reflecting both ecological variability (soil pH and climate variables) and uncertainty in coefficient estimates, with the latter calculated from the 2.5th and 97.5th percentiles of 10,000 Monte Carlo simulations of coefficient uncertainty. ^cAverage of the site-specific CL % errors, calculated from the lower and upper endpoints of the 95% confidence interval of Monte Carlo simulations of coefficient uncertainty repeated at each site.

Furthermore, when we calculate critical load estimates (Table 2) for specific sites using our national-scale equations (Table 1) we find that they are consistent with experimental data from long-term N additions. Our critical load estimate of $8.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for grassland at the Cedar Creek LTER site is consistent with the critical loads estimated there using statistical extrapolation of results from a fertilization experiment (95% inverse prediction interval of $7.3\text{-}15.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (10). Likewise, our estimated critical load of $11.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for forest in the Fernow Experimental Forest is consistent with the interpretation (27) that ambient N deposition already exceeded critical loads prior to the initiation of experimental additions at Fernow. This consistency of experimental and gradient results strengthens our confidence in our critical loads estimates for sites without long-term experimental data.

Finer-scale N Deposition Gradients within Specific Vegetation Communities. Having just demonstrated relationships between plant species richness and N deposition at a national scale, we now shift our focus to the community scale at which many local

land management activities are directed. Within community-scale deposition gradients we again found that relationships between plant species richness and N deposition were often conditional on soil and climate covariates. Plant species richness declined as N deposition increased in 36.5% of the 44 studied gradients (16% unconditional, 20.5% conditional on a covariate), increased with N deposition in 18% of the gradients (4.5% unconditional, 13.5% conditional), and showed no relationship with N deposition in 45.5% of gradients (Fig. 3). Most of the gradients where species richness increased with N deposition had N deposition averaging $3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ or less (Fig. 4). Overall, plant species richness was more likely to decline with increasing N deposition along gradients with more acidic soil conditions (Fig. 4a), or warmer (Fig. 4b), wetter (Fig. 4c) climates, broadly consistent with the national analysis. Both the community-level and national-level analyses showed decreases in more acidic conditions, and while the community-level analysis showed declines under warmer conditions, that relationship was only present for open canopy systems for the national analysis. This restricted gradient analysis was only possible in the subset of vegetation types that spanned an

409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476

477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544

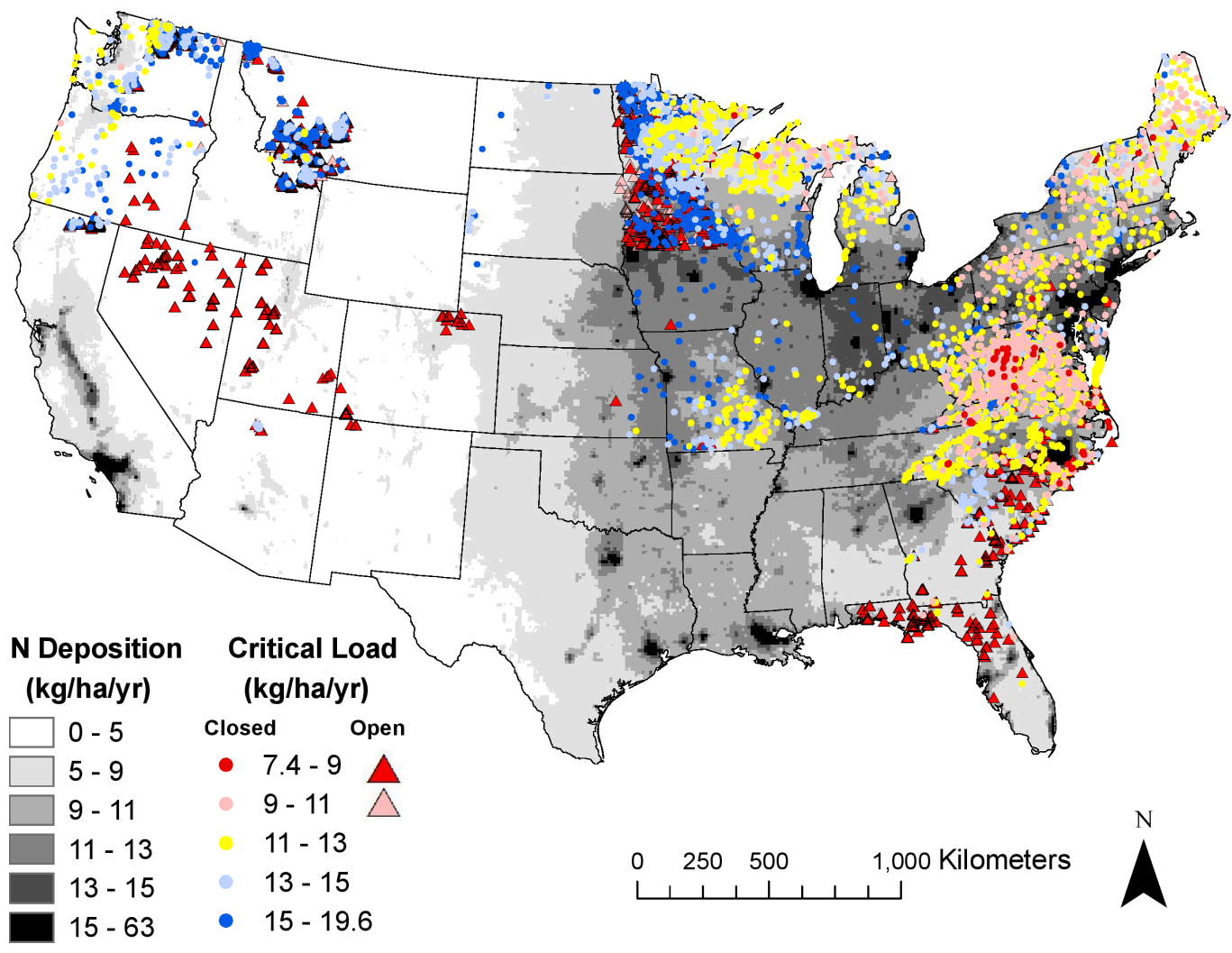


Fig. 2. Nitrogen deposition (gray-scale) and critical loads for nitrogen deposition based on total graminoid plus forb species richness (colored symbols). The 3,317 open sites (combined grassland, shrubland, and woodland vegetation types) are portrayed with triangles and the 11,819 closed canopy sites (deciduous, evergreen, and mixed forests) are portrayed with circles. Background deposition values are the average of 27 years of wet deposition (NADP 1985-2011) plus the average of 10 years of dry deposition (CMAQ 2002-2011). Other variation in critical loads is due to the other predictor variables (soil pH, temperature, and precipitation).

adequate N deposition range (Table S3), but its power lies in the capacity to detect relationships missed by national-scale analyses, and the restriction to datasets within similar methodologies and vegetation types to control for any potential spurious relationships.

We demonstrate the context-dependency of N deposition effects using the three forested vegetation types (*Acer-Betula* alliances, *Quercus alba* alliances, *Pseudotsuga menziesii* alliances) that were represented in more than three separate gradients (Table S5). In these cases, species richness declines were more readily detected where precipitation and temperature were highest, or where N deposition reached or exceeded 7.5-9.5 kg ha⁻¹ yr⁻¹. Among the four *Acer - Betula* forest gradients, only the gradient with the highest precipitation and temperature showed an unconditional species richness decline with N deposition. Among the six *Quercus alba* forest gradients, only the two gradients where N deposition was always greater than 9.5 kg ha⁻¹ yr⁻¹ showed a species richness decline with N deposition. Finally, among the four *Pseudotsuga menziesii* forest and woodland gradients, we observed increases in richness in the three gradients where deposition was always below 4.6 kg ha⁻¹ yr⁻¹, but in the gradient with up

to 7.5 kg ha⁻¹ yr⁻¹ a species decline emerged. Shifts in relationships for the same vegetation type along different N deposition ranges are consistent with the curved response surfaces illustrated in Fig. 1.

In grasslands and shrublands, we hypothesized that the competitive exclusion mechanism of N deposition-induced species loss would be strong since there is greater potential for some herbaceous species to shade or grow faster than other non-shade-tolerant or slower-growing herbaceous species. Consistent with this hypothesis, one of three shrubland gradients showed an unconditional decrease in plant species richness with increasing N deposition, even though all shrubland gradients experienced N deposition of 5 kg ha⁻¹ yr⁻¹ or less (Table S5). Shrubbylands experiencing higher N deposition have shown even stronger responses (e.g., native species richness declines in coastal sage scrub with N deposition beyond 8.7 kg ha⁻¹ yr⁻¹ (28)). Grassland species richness declined once N deposition exceeded 8 kg ha⁻¹ yr⁻¹ (*Schizachyrium scoparium - Bouteloua curtipendula* and *Andropogon gerardii - Sorghastrum nutans* grasslands in Table S5), consistent with experimental work (10) and a continental-scale study of European grasslands (11).

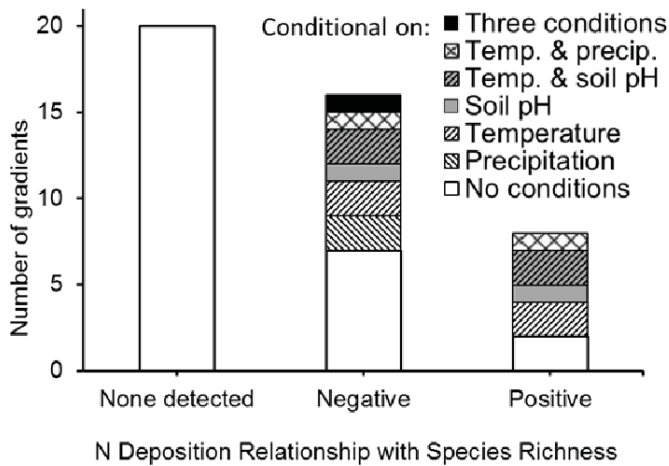


Fig. 3. Summary of relationships between plant species richness and N deposition in 46 gradients. Gradients (uniquely defined by vegetation type and data source) contain 6,807 sites, conditional on soil pH, average annual temperature, annual precipitation, and N deposition interactions with each of the other three predictors. In conditionally negative or positive gradients the relationship was either negative or positive, respectively, for more than half of the range of the moderating variable(s).

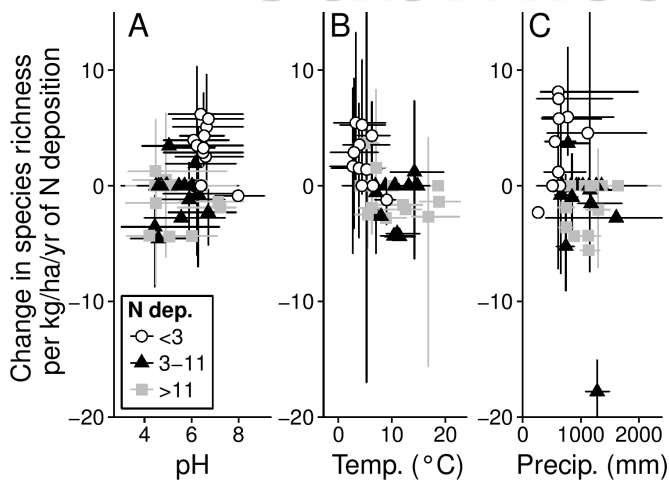


Fig. 4. Magnitude of plant species richness changes associated with N deposition, as moderated individually by (a) soil pH, (b) average temperature, or (c) annual precipitation. Each point, symbolized by the mean N deposition of that gradient ($\text{kg ha}^{-1} \text{yr}^{-1}$), represents an individual gradient with a single narrow vegetation type. Species richness change is calculated as the simple slope of nitrogen deposition from multiple regression coefficients: $\beta_N + (\beta_{N \times M} \times M_i)$, where β_N is the parameter for N deposition, $\beta_{N \times M}$ is the parameter for the interaction of N deposition and the moderating variable M, and M_i are the mean (symbol) and range (lines) of the moderating variable M across the gradient. Unlike in Fig. 3, each predictor variable is considered separately.

Scale- and Context-dependency of Species Richness Relationships with N Deposition. Our results demonstrate that negative relationships between N deposition and species richness are common, albeit not universal, and that fine-scale processes appear to moderate vegetation responses to N deposition in many areas. This is consistent with the mechanisms of biodiversity loss described above (4, 29), all of which may operate simultaneously in ecosystems. At both the national and fine scales, we identified environmental conditions where there was little to no relationship between N deposition and species richness, and conditions under which N deposition increases species richness, which helps place previous work (30, 31) in context, and unifies these conflicting empirical results to ecological theory. We were able to identify

N deposition relationships with species richness by accounting for heterogeneous soil pH and climate factors within distinct vegetation types. As national-scale, high-resolution datasets for other covariates such as herbivory (32) and disturbance history become available it should be possible to resolve in even finer detail this relationship between N deposition and plant species richness.

Conclusion

Our continental-scale analysis found that the threat of N deposition to herbaceous plant species richness is ecosystem-specific, with some ecosystems more vulnerable than others, and some conditions conferring greater vulnerability. Ecosystems with open vegetation (grasslands, shrublands, and woodlands) had lower critical loads of N deposition ($7.4\text{-}10.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) than ecosystems with closed-canopy forest vegetation ($7.9\text{-}19.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Within these broad vegetation groups, declines in species richness along gradients of increasing N deposition were more likely to occur in ecosystems with more acidic soils. Climate also interacted with N deposition to help explain species richness, but its influence was less consistent across scales. Increasing the number of N-addition experiments with treatment levels spanning 2-20 $\text{kg ha}^{-1} \text{ yr}^{-1}$ and implementing them across the full range of soil pH, climate, and vegetation types that exist on the landscape would be a very welcome complement to the correlative work that we have reported here. In the meantime our work suggests that the mechanism of competitive exclusion via shading is likely of reduced strength in the comparative shade of forest understories, whereas the acidification and competitive exclusion mechanisms are probably more likely to occur synergistically in the high-light environment characteristic of grasslands. We successfully identified ecosystems vulnerable to N deposition and refined herb-based N deposition critical loads (18) by incorporating a broad range of vegetation types, N deposition loads, soil substrates, and climate conditions in our analysis. This identification of vulnerable ecosystems and influential environmental factors is critical for managers to set monitoring and conservation priorities.

Methods

Data acquisition and management. We compiled vegetation data from multiple sources (Table S1) since a single standardized national dataset of herbaceous plant species presence and abundance with sufficient spatial coverage and plot density is not available for the United States. We retained only terrestrial sites sampled after 1989 that had a complete inventory of species from graminoid and forb functional groups, quantitative abundance for each plant species, a sampling area of 100-700 m^2 , and known geographic coordinates. At each site we calculated total herbaceous (defined here as forbs and graminoids) plant species richness, a conservative measure since total richness could remain unchanged even as invasive species richness increases and native species richness declines.

We estimated N deposition by adding CMAQ (Community Multiscale Air Quality) model dry deposition estimates to interpolated NADP (National Atmospheric Deposition Program) wet deposition and extracting a value based on coordinates for each site. The CMAQ version 5.0.2 dry deposition estimate was a 10-year average (2002-2011) with 12-km resolution, using models run in 2014 by Robin Dennis at EPA. CMAQ dry deposition estimates, or other comparable estimates with fine resolution, are not yet available at a national scale before 2002. The NADP wet deposition was a 27-year average (1985-2011), which we resampled from the raw 2.33833-km resolution to the 4-km resolution of the PRISM precipitation data that had been used in the interpolation.

We extracted climate covariates, specifically average annual precipitation and temperature from 30-year PRISM climate normals (1981-2010) and obtained soil pH, where available, from the same datasets that supplied vegetation data. If soil data from soil samples co-located with vegetation data were not available, then pH from 1:1 water extracts from the national USDA SSURGO soil dataset was used. We retained the 15,611 sites with non-missing species richness and predictor values that met the criteria for analyses at either the national-scale (data sources combined but plots filtered based on area) or gradient-scale (data sources considered separately) that are described below.

Data analysis. For our initial national-scale analysis we began with all 15,136 sites and then, based on expected differences in mechanisms, we divided those sites into two broad vegetation types, namely closed canopy

(deciduous forest, evergreen forest, and mixed forest) and open canopy (grassland, shrubland, and woodland) vegetation types. Within each of these two groups we determined the relative importance of our four primary predictor variables (N deposition, soil pH, precipitation, and temperature) by looking at the R^1 coefficients of determination (based on absolute deviations in quantile regression rather than squared deviations) of b-spline models with and without these four main effects. Next we examined non-linear regressions of the 0.50 (median), 0.10, and 0.90 quantiles of total herbaceous plant species richness response to N deposition (quadratic), soil pH, mean annual temperature, annual precipitation, and the two way interactions involving N deposition (i.e., N x precipitation, N x temperature, and N x soil pH) using the quantreg package of R (version 3.0.2) software. Out of all possible models, we selected the model with the lowest AICc (corrected Akaike information criterion) for each of the two broad vegetation types (Table 1, Fig. 1).

We used the median quantile regression model with the best AICc to calculate separate critical loads of N deposition for open and closed canopy vegetation. Qualitatively, critical loads of N deposition are defined here as the N deposition threshold at which species richness begins to decline, corresponding graphically to the N deposition level at which a hump-shaped relationship between N deposition and species richness reaches its peak value of species richness. Quantitatively, we took the first derivative of the best model with respect to nitrogen, and set that expression to an inequality of less than zero. That leads to an expression for change in species richness that are negative (i.e. losses of species), as they relate to the potential factors in the best statistical model (e.g. N deposition, pH, temperature, precipitation). Solving that expression for N gives an expression for values of N deposition that lead to losses of diversity, which we are using as an estimate of the critical load. For critical loads specific to each site, we used the coefficients from the critical load expression and site-specific covariate values. We subtracted critical loads from N deposition to determine exceedances of N deposition critical loads. Three sets of exceedances were calculated, utilizing 1) the median point estimates of critical loads, as well as the 2) upper and 3) lower limits of the 95% CI of the critical loads. Only

the exceedances based on the median point estimates of critical loads are presented graphically and in the abstract.

Further community-scale analyses were focused on individual alliances as defined by the National Vegetation Classification (NVC) (33). We analyzed alliances with deposition gradients with maximum N deposition that was either 2.5 times or $4 \text{ kg ha}^{-1} \text{ yr}^{-1}$ greater than minimum N deposition, and that had at least 20 sites from at least one common data source. These gradient criteria reduced the number of sites to 6,807. For each N deposition gradient, we performed multiple regressions of species richness against N deposition, with the same predictor variables and the same model selection procedure as in the national analysis (except that N deposition was only first order).

This article contains supporting information online at www.pnas.org/lookup/suppl. Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.xxxxx>

Acknowledgments. This paper arose from the "Diversity and Nitrogen Deposition" working group supported by the John Wesley Powell Center for Analysis and Synthesis, funded by the U.S. Geological Survey. The U.S. Environmental Protection Agency (contract EP-12-H-000491) and the CESU Network (National Park Service grant P13AC00407 and U.S. Geological Survey grant G14AC00028) provided additional funding. Vegetation data shared by: USFS FIADB Vegetation Indicators Program, ESA's Vegbank, Minnesota DNR's Biological Survey Program, New York, Virginia, and West Virginia Natural Heritage Programs, Robert Peet and the Carolina Vegetation Survey, NPS's SCPN, University of Wisconsin PEL, Kevin Knutson of USGS, and co-authors. The USGS supports the conclusions of research conducted by their employees, and peer-reviews and approves all of their products consistent with USGS Fundamental Science Practices. The views expressed in this manuscript do not necessarily reflect the views or policies of the US Environmental Protection Agency or the USDA Forest Service. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. The authors declare no competing financial interest.

- Galloway JN, *et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70(2):153-226.
- Porter EM, *et al.* (2013) Interactive effects of anthropogenic nitrogen enrichment and climate change on terrestrial and aquatic biodiversity. *Biogeochemistry* 114(1-3):93-120.
- Sala OE, *et al.* (2000) Biodiversity - Global biodiversity scenarios for the year 2100. *Science* 287(5459):1770-1774.
- Bobbink R, *et al.* (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20(1):30-59.
- Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 441(7093):629-632.
- Isbell F, *et al.* (2013) Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences of the United States of America* 110(29):11911-11916.
- Isbell F, *et al.* (2011) High plant diversity is needed to maintain ecosystem services. *Nature* 477(7363):199-202.
- De Schrijver A, *et al.* (2011) Cumulative nitrogen input drives species loss in terrestrial ecosystems. *Global Ecology and Biogeography* 20(6):803-816.
- Clark CM, *et al.* (2007) Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters* 10(7):596-607.
- Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451(7179):712-715.
- Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science* 303(5665):1876-1879.
- Stevens CJ, *et al.* (2010) Nitrogen deposition threatens species richness of grasslands across Europe. *Environmental Pollution* 158(9):2940-2945.
- Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. *Science* 324(5927):636-638.
- Neufeld HS, Young DR (2014) Chapter 3: Ecophysiology of the herbaceous layer in temperate deciduous forests. *The Herbaceous Layer in Forests of Eastern North America*, ed Gilliam FS (Oxford University Press, New York), 2nd Ed, pp 34-95.
- Beier CM, *et al.* (2012) Changes in faunal and vegetation communities along a soil calcium gradient in northern hardwood forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 42(6):1141-1152.
- Hall SJ, *et al.* (2011) Ecosystem response to nutrient enrichment across an urban airshed in the Sonoran Desert. *Ecological Applications* 21(3):640-660.
- Ladwig LM, *et al.* (2012) Above- and belowground responses to nitrogen addition in a Chihuahuan Desert grassland. *Oecologia* 169(1):177-185.
- Pardo LH, *et al.* (2011) Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecological Applications* 21(8):3049-3082.
- Bobbink R, Hicks WK (2014) Chapter 14: Factors affecting nitrogen deposition impacts on biodiversity: An overview. *Nitrogen deposition, critical loads, and biodiversity*, eds Sutton MA, Mason KE, Sheppard LJ, Sverdrup H, Hauber R, Hicks WK (Springer, Dordrecht), pp 127-138.
- Stevens CJ, Thompson K, Grime JP, Long CJ, Gowing DJG (2010) Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. *Functional Ecology* 24(2):478-484.
- Chen DM, Lan ZC, Bai X, Grace JB, Bai YF (2013) Evidence that acidification-induced declines in plant diversity and productivity are mediated by changes in below-ground communities and soil properties in a semi-arid steppe. *Journal of Ecology* 101(5):1322-1334.
- Sverdrup H, *et al.* (2012) Testing the feasibility of using the ForSAFE-VEG model to map the critical load of nitrogen to protect plant biodiversity in the Rocky Mountains region, USA. *Water Air and Soil Pollution* 223(1):371-387.
- Bowman WD, Gartner JR, Holland K, Wiedermann M (2006) Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: Are we there yet? *Ecological Applications* 16(3):1183-1193.
- Diekmann M, *et al.* (2014) Long-term changes in calcareous grassland vegetation in North-western Germany - No decline in species richness, but a shift in species composition. *Biological Conservation* 172:170-179.
- Magurran AE (2004) *Measuring biological diversity* (Blackwell Publishing, Malden, MA) p 256.
- Roth T, Kohli L, Rihm B, Achermann B (2013) Nitrogen deposition is negatively related to species richness and species composition of vascular plants and bryophytes in Swiss mountain grassland. *Agriculture, Ecosystems and Environment* 178:121-126.
- Gilliam FS (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. *Journal of Ecology* 94(6):1176-1191.
- Fenn ME, *et al.* (2010) Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. *Journal of Environmental Management* 91(12):2404-2423.
- Suding KN, *et al.* (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America* 102(12):4387-4392.
- Verheyen K, *et al.* (2012) Driving factors behind the eutrophication signal in understory plant communities of deciduous temperate forests. *Journal of Ecology* 100(2):352-365.
- Dirnböck T, *et al.* (2014) Forest floor vegetation response to nitrogen deposition in Europe. *Global Change Biology* 20:429-440.
- Throop HL, Lerdau MT (2004) Effects of nitrogen deposition on insect herbivory: Implications for community and ecosystem processes. *Ecosystems* 7(2):109-133.
- Grossman DH, *et al.* (1998) International classification of ecological communities: terrestrial vegetation of the United States. Volume I: The National Vegetation Classification System: development, status, and applications. (The Nature Conservancy, Arlington, VA).
- Lamarque JF, *et al.* (2013) Multi-model mean nitrogen and sulfur deposition from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): evaluation of historical and projected future changes. *Atmospheric Chemistry and Physics* 13(16):7997-8018.
- Schwede DB, Lear GG (2014) A novel hybrid approach for estimating total deposition in the United States. *Atmospheric Environment* 92:207-220.
- Dengler J, *et al.* (2012) Special Volume: Vegetation databases for the 21st century. - Biodiversity & Ecology p 447.
- Knutson KC, *et al.* (2014) Long-term effects of seeding after wildfire on vegetation in Great Basin shrubland ecosystems. *Journal of Applied Ecology* 51(5):1414-1424.
- Dennis RL, *et al.* (2013) Sensitivity of continental United States atmospheric budgets of oxidized and reduced nitrogen to dry deposition parametrizations. *Philosophical Transactions of the Royal Society B-Biological Sciences* 368(1621-20130124):1-10.
- Daly C, *et al.* (2008) Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. *International Journal of Climatology* 28(15):2031-2064.