4 METHODS

L.H. Pardo, E.A. Lilleskov, L.H. Geiser, M.J. Robin-Abbott

4.1 Approach

We present an up-to-date review of current knowledge relating nitrogen (N) inputs to ecological effects, from published literature, reports, and some recent unpublished studies. Our goal is to summarize research on ecosystems across the United States. We include N gradient, long-term, and fertilizer studies; for fertilizer studies, we focus on those with low N additions (i.e., close to ambient deposition). We include both publications that report a response and those that report the lack of response.

Empirical critical loads are determined by recording the deposition level at which detrimental ecological effects have occurred in the field. The lowest deposition level at which a detrimental response occurs is considered the critical load. In cases where there is a variation in the level of N input that causes a given response, a range for the critical load is often reported (e.g., Bobbink et al. 2003). Empirical critical loads are useful because they can be used to determine the critical load based on the best available information for an ecosystem type when data are not available at a given site. Under the best circumstances, empirical critical loads link observed ecosystem response to deposition.

Disadvantages of empirical critical loads stem from their reliance on observed cause-and-effect responses rather than an understanding of a process or mechanism. Because the observed responses to a given deposition level are based on past deposition scenarios, they may not be indicative of the response that might occur in the future. For example, if a response is observed to a given rate of deposition (or fertilization) after a certain number of years, it is possible that a lower deposition over a longer time period would cause the same detrimental effect. For critical loads derived from gradient studies, generally, the assumption is that the N deposition rate is the major factor contributing to changes in a particular response (e.g., foliar N concentrations, species richness) along the gradient, even though soil, climate, and biogeochemical factors may also influence the response. For lichens (see section 4.3), these factors were taken into account. For critical loads derived from fertilization studies, the effects of added N are isolated through controlled experimentation; however, the scale of these experiments is often fairly small and their numbers are limited, making extrapolation to the ecoregion level difficult. Another pitfall of the empirical approach is that the observed response may be unique to the site at which it was measured (because of location-specific disturbance history or site characteristics such as soil thickness) and may not be truly representative of the ecosystem type. Observations used to determine or estimate empirical critical loads are generally made over a relatively short term. In contrast, the residence time of N within major ecosystem compartments (e.g., soil, sediment, forest biomass) is generally on the order of decades to centuries. As a result, empirical critical loads provide a limited understanding of true ecosystem response to sustained elevated N inputs or their response to changes in these inputs. Critical loads dynamic modeling addresses some of the disadvantages of the empirical critical loads approach. However, current modeling capabilities do not enable large scale mechanistic modeling of critical loads for ecoregions across the United States. Thus we utilize multiple sources of current empirical information (multiple gradient and fertilization studies) to determine critical loads. To address these uncertainties, we present the ranges of responses observed. Where we have estimated an empirical critical load for N, we have made some assessment of the reliability of that estimate. We used the same categories for reliability as those used in European assessments (Bobbink et al. 2003, UBA 2004):

- reliable ##: when a number of published papers of various studies show comparable results
- fairly reliable #: when the results of some studies are comparable
- expert judgment (#): when very few or no empirical data are available for this type of ecosystem. The N critical load is then based

> upon expert judgment and knowledge of ecosystems, which are likely to be more or less comparable with this ecosystem.

In all cases, accurate estimates of N deposition are essential for developing useful empirical critical loads of N.

In presenting the ecosystem responses and critical loads, we identify the response parameter and the threshold. Within an ecosystem, there could be very different types of response parameters; hence the critical load or input which induces each response may vary considerably. This poses a challenge in setting a critical load for the ecosystem. In some cases, it may be appropriate simply to choose the lowest critical load value, while in other cases, there may be some responses more characteristic or of greater concern which should be considered the primary indicator for that ecosystem. Furthermore, comparing critical loads that were set using different critical responses presents a challenge.

Several criteria should be determined before assigning empirical critical loads to an ecosystem type. There are four key criteria to consider: what response variables are relevant, what types of studies are acceptable, how to choose the value for the critical load, and if and when we can extrapolate conclusions beyond the study area.

Approaches for setting empirical critical loads for N for specific ecosystem components are discussed below. Approaches for setting critical loads for aquatic ecosystem components are presented in Chapters 17 and 18.

4.2 Mycorrhizal Fungi

4.2.1 Response Variables

The impacts of N deposition on arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) have been determined primarily by examining the changes in percent root colonization, soil spore density (AMF) or sporocarp production (EMF), spore or sporocarp species composition, DNA or immunologic assays of species composition and structure on root tips or of hyphae in soils, and functional analysis of fungal communities.

Data from naturally occurring, anthropogenic N deposition gradients have been particularly useful in determining critical loads because of the ability to fit response curves. In deciding if N inputs are affecting mycorrhizal fungi, we focused mostly on community response (species richness and community structure changes) and functional responses. However, where these are lacking, we considered biomass responses, giving them less weight than the other factors.

4.2.2 Acceptable Studies

For information sources, we put more weight on results of long-term (>10 yr) fertilizations and gradient studies rather than short-term fertilizations, as the latter are unlikely to represent a steady-state response to N. Gradients have the advantage over typical two-level experiments in that response curves and threshold responses can be derived. However, even a two-level study with only a control and treatment can put an upper bound on critical loads if there is a response and lower bound if there is no response. Therefore we have included some data from experimental sources, recognizing their limitations.

4.2.3 How the Critical Load Value was Chosen

For our purposes, any detectable deviation in mycorrhizal community structure or composition from the baseline state is considered to have crossed a critical load threshold. For example, if the best-fit response curve in an N deposition gradient indicates a linear response starting from the baseline, then any addition above the baseline would exceed the critical load. If, in contrast, the data suggest a nonlinear response with a threshold below which no directional community change can be detected, then the critical load is set at this threshold. The criterion of any detectable directional change is a very strict criterion that could be open to debate, but it has the merit of being relatively easy to define.

One justification for using the community change criterion is that there appear to be functional consequences for host plants arising from N-driven community change. This has been determined by growing plants using inoculum that has been subject to elevated N. Nitrogen-affected inoculum not only has lower spore density, but also has lower diversity and shifts in species composition (Egerton-Warburton and Allen 2000, Egerton-Warburton et al. 2007, Sigüenza et al. 2006). Growth response studies showed that native late successional plants had reduced biomass with N-affected inoculum, while early successional plants were unaffected or sometimes had increased biomass. This indicates that long-term trends in losses of late seral (successional) vegetation and replacement by early seral or invasive species were in part promoted by shifts in mycorrhizal fungal species composition due to elevated N deposition (Corkidi et al. 2002, Sigüenza et al. 2006).

4.2.4 Extrapolation

For ecological regions for which there are no data, we have cautiously extrapolated our critical load values to regions that have similar biota, climate, and soils. For example, we have suggested that ectomycorrhizal community response to N in an Engelmann spruce forest in the Rockies (Northwestern Forested Mountains ecoregion), for which there are no data of which we are aware, would be similar to that of communities in Alaskan white spruce (Marine West Coast Forest ecoregion) and northeastern red spruce-balsam fir (Northern Forest ecoregion) forests, for which we do have data. Thus we have used, with qualification, the critical loads for the latter two as the basis for a provisional estimate for the critical load for the former.

4.3 Lichens and Bryophytes

Lichens and bryophytes are among the most sensitive bioindicators of N in terrestrial ecosystems (Blett et al. 2003, Bobbink et al. 2003, Fenn et al. 2003, Vitt et al. 2003). Consequently, the implementation of critical loads protective of the most sensitive lichens and bryophytes is likely to prevent undesired impacts to the broader terrestrial ecosystem by also protecting less N-sensitive vegetation. Protecting the integrity of lichen and bryophyte communities, moreover, ensures preservation of their contribution to biological diversity and their varied ecological roles in nutrient cycling, hydrological flux, and supply of wildlife forage and nesting materials (McCune et al. 2007).

Lichens, especially forest epiphytes, have been systematically surveyed for air quality monitoring purposes by the U.S. Forest Service Forest Inventory Analysis (FIA) program and other U.S. Forest Service and National Park Service efforts, and many of these data are available electronically: the NPLichen database (Bennett and Wetmore 2005a, 2005b; U.S. Geologic Survey 2010), the NPElement database (U.S. Geologic Survey 2009), the U.S. Forest Service Forestry Inventory and Analysis Program (U.S. Forest Service 2005, 2007), and the U.S. Forest Service National Lichens and Air Quality Database (U.S. Forest Service n.d.). No equivalent efforts exist for bryophytes in the United States. Therefore, apart from the Tundra and Taiga chapters (Chapters 5 and 6) (which extrapolate some critical loads from European and Canadian studies) and the Desert chapter (Chapter 12) (in which bryophytes are integral components of cryptogamic crusts), we primarily reference lichen studies.

4.3.1 Terminology

Lichens are adapted to different levels of N availability and most species can be sorted into one of three N indicator groups: *oligotrophs* are more or less restricted to nutrient-poor environments; *mesotrophs* have an intermediate nutrient requirement; and *eutrophs* thrive in nutrient-enhanced environments. A few lichens have broad ecological amplitudes and tolerate a large range of N availability. As anthropogenic N is added to background N levels, the excess N favors the small, cosmopolitan eutrophs at the expense of native oligotrophic and mesotrophic forage and N_2-fixing lichens (McCune and Geiser 2009).

To avoid possible confusion from variable terminology in the literature, we consistently used the broad terms ‘oligotroph’, ‘mesotroph’ and ‘eutroph’ in lieu of the more classic lichenological terms, ‘acidophyte’, ‘neutrophyte’, and ‘nitrophyte or nitrophile’, respectively. This is necessary because most North American studies of N deposition effects have not considered substrate pH, deposition pH, or N source when classifying lichens to N indicator groups. Because low-fertility environments are frequently dominated by acidic conifer substrates, and moderate- to high-fertility environments generally include more hardwoods with more neutral bark and more nutrient-enriched canopy drip, the classic and broad terms usually correspond to similar lichen lists (Jovan 2008, Geiser et al. 2010)

Common names for lichens follow the Natural Resources Conservation Service PLANTS database (NRCS 2011). Because many lichen common names in PLANTS are not unique, the scientific name and authority are specified when a common name is used for the first time within a chapter. Scientific nomenclature follows Esslinger (2010).

4.3.2 Response Variables

To identify critical loads for the Marine West Coast Forests, Northwest Forested Mountains, and Mediterranean California ecoregions, we used two epiphytic lichen responses that are closely linked to ecological impact: N concentration in lichen thalli, and composition of lichen communities. Changes in thallus N concentrations and physiology usually precede measurable changes in lichen community composition. Local N deposition is correlated with N concentrations of lichen thalli, which passively accumulate N in excess of nutritional requirements (Bruteig 1993, Søchting 1995). This response is typically linear at low N deposition levels (Glavich and Geiser 2008). The upper limit of thallus N concentrations among clean sites is typically used as the response threshold. Increasing N deposition, likewise, causes changes in lichen community composition—such as a decrease in the number of oligotrophic species and an increase in the number of eutrophic species.

For the Taiga, Tundra, and North American Desert ecoregions, we included physiological and morphological measures of lichen status, such as changes in photosynthetic capacity (e.g., chlorophyll pigment ratios, carbon dioxide (CO_2) uptake), membrane integrity, amounts of characteristic protective chemicals (e.g., usnic acid), apportionment of resources to algal vs. fungal partners, ultrastructural integrity, or growth rates.

4.3.3 Acceptable Studies

As with the mycorrhizal fungi, we primarily relied on gradient studies as information sources rather than short-term fertilizations. In addition, studies of epiphytes are considered superior to studies of ground-dwelling species due to epiphytes' greater independence from soil nutrients. For the Rocky Mountain, Marine West Coast Forests, and Mediterranean California ecoregions, we were fortunate to obtain community data from regional, systematic surveys of epiphytic lichens, primarily conducted by the FIA program. Most of the physiological studies were N-addition studies. Since they were short-term additions or added N to already elevated background deposition levels, we feel they do not adequately predict long-term effects. However, they do prescribe upper limits for critical loads.

4.3.4 How the Critical Load Value was Chosen

We determined the critical load by identifying the level of N deposition which led to specific ecological impacts (changes in community composition and increased thallus N concentration) using the datasets described above. The ecological impact at any N deposition level was quantified by doing a regression on N deposition against proportions of oligotrophic, mesotrophic, and eutrophic species, or an index called the "air score" (Geiser and Neitlich 2007, Jovan 2008). The value of N deposition at the ecological threshold is the critical load. The ecological thresholds were community shift toward a higher proportion of eutrophic species and elevated thallus N concentration. The exact value for the threshold thallus N concentration was typically set based on the 97.5 percent quantile for clean sites. The 97.5 percent quantile is the thallus N concentration below which 97.5 percent of clean site thallus N concentrations fall. The exact threshold for species composition varied because the baseline (species composition at an unpolluted site) varied in different ecosystems (see Table 4.1 and section 4.3.5). Because all the lichen response measures were linear with respect to deposition, we favor response thresholds high enough to allow for natural variability across clean sites, but low enough to protect communities from a shift in dominance of indicator groups. We used the 95 percent confidence interval as the range for the critical load.

Forest Inventory and Analysis (FIA) Program lichen-community based "air scores" are a measure of air quality generated from lichen community data and

Table 4.1—The input variables, assumptions, and rationale behind the calculation of lichen critical loads for U.S. ecoregions, derived from the Marine West Coast Forest model: Critical load $kg\ N\ ha^{-1}\ yr^{-1}$= (0.0918 + 0.0024 [mean annual precipitation] + threshold air score)/0.1493. Precipitation is estimated from PRISM 1961-1990 annual average precipitation maps; lichen response thresholds are based on expert opinion (Geiser et al. 2010).

	Mean ann. precip. (cm)			Biological response threshold		
Ecoregion	Min	Max	Air score	Minimum % oligotrophs	Maximum % eutrophs	Rationale/Assumptions
Taiga (US)	20	80	0.02-0.21	41	27	Acidic coniferous substrates, cold temperatures favor oligotrophs. Therefore, community response threshold set with comparatively high oligotroph and low eutroph composition.
Northern Forests (US)	100	240	0.21	30-41	27-34	Climate and forest type similar to Marine West Coast Forest. Therefore, use same threshold. Synergistic or confounding effects of acid deposition of S and N, which is much greater here than Marine West Coast Forest, is not considered in this analysis.
Northwestern Forested Mountains: Alaska	30	80	0.02-0.21	41	27	Lower thresholds needed for Alaska because of cooler temperatures (see Taiga rationale).
Marine West Coast Forests	44	451	0.21	30-41	27-34	Model was developed for this region by Geiser et al. (2010)
Northwestern Forested Mountains: non-Alaska	30	203	0.21-0.49	25-40	27-47	Lower and upper thresholds calculated by Fenn et al. 2008. Low humidity and high temperatures favorable to eutrophs and unfavorable to oligotrophs except in high elevation fog zones.
Eastern Temperate Forest: eastern hardwoods	71	305	0.33	25-30	34-47	Humid climate favors oligotrophs but hardwood dominance favors mesotrophs and eutrophs, so response threshold allows comparatively more eutrophs. Synergistic or confounding effects of acid deposition of S and N are not considered in this analysis.
Eastern Temperate Forest: southeast Coastal Plain	102	178	0.21-0.33	30	34	Humid climate and acidic conifer substrates favor oligotrophs. Warm summer temperatures limiting to oligotrophs? Synergistic or confounding effects of acid deposition of S and N are not considered in this analysis.
Mediterranean CA: mixed Conifer Forest	41	127	0.33-0.49	25	47	Climate and forest type closest to Northwestern Forested Mountains--used Fenn et al. 2008 upper threshold to account for comparatively warmer, drier conditions compared to the Sierras.
Mediterranean CA: Chaparral and Central Valley	17	156	0.33-0.49	25	47	Threshold (50% eutrophs) selected from overlay of CMAQ data on Jovan and McCune 2006 analysis of this ecoregion. Hot dry summers, hardwood dominance favors mesotrophs and eutrophs.
Temperate Sierras	30	178	0.49	21-25	47-57	Natural contribution of eutrophs to lichen community composition presumed relatively high due to dry, hot climate.

region-specific interpretive models separating air pollution effects on community composition from climate, elevation and other site effects (Jovan 2008). Models are typically developed from a calibration subset of regional FIA lichen community data using nonmetric multidimensional scaling and Sorenson's distance measure (McCune and Mefford 1999) to ordinate sites in N-dimensional space. With the aid of overlays, regression, scatter plots and other tools, at least two strong gradients can usually be extracted from the ordination: one relating to air quality and a second relating to macroclimate. Air quality and climate are then scored as the distance of the community (site) along the respective gradient. Repeat or additional surveys can be fitted and scored at any time to assess spatial and temporal trends. Finished models are available for the southeastern United States (McCune et al. 1997), California's greater Sacramento Valley (Jovan and McCune 2005) and Sierra Nevada (Jovan and McCune 2006), and western Oregon and Washington (Geiser and Neitlich 2007). In the western United States, nutrient N deposition is considered the dominant air pollutant affecting lichen communities (Jovan 2008, Geiser et al. 2010); in the southeastern United States, N- and S-containing acidifying and fertilizing compounds are considered most influential (McCune et al. 1997).

Lichen community and thallus N concentration appear to correlate better with N concentration (mg L^{-1}) in deposition as opposed to N load (kg ha^{-1} yr^{-1}) (Geiser et al. 2010). Because lichen thalli accumulate and leach N in dynamic equilibrium with concentrations of pollutants in precipitation, precipitation can have a diluting effect on the concentrations of pollutants to which lichens are exposed. Thus, the same load of N deposited in a higher volume of precipitation will have a smaller impact. Therefore, we accounted for precipitation volume when developing lichen critical loads based on measurements of bulk (NADP-style) or modeled N deposition.

In the Marine West Coast Forests, Northwest Forested Mountains, and Mediterranean California ecoregions, lichen-based critical loads were derived by correlating lichen responses (described above) to Interagency Monitoring of Protected Visual Environments (IMPROVE[2]; fine particulate ammonium nitrates (NH_4NO_3) and sulfates ($(NH_4)_2SO_4$) in air) and NADP[3] monitoring networks (wet deposition of inorganic N), 10 year total annual average deposition of 16 N-species modeled by CMAQ[4], or *in-situ* deposition measurements of ammonium (NH_4^+) and nitrate (NO_3^-) in bulk and canopy throughfall.

4.3.5 Extrapolation

To make initial estimates of lichen-based critical loads for N in forested ecological regions where existing analyses were lacking, (i.e., Taiga, Northern Forests, Eastern Forests, and the Temperate Sierras), and to encompass more of the Northwest Forested Mountains ecoregion, we applied a model that was developed in western Oregon and Washington for the Marine West Coast Forests ecoregion (Geiser et al. 2010). This model has been shown to replicate previously published critical loads for wet oak woods of Scotland,

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[2] IMPROVE (Interagency Monitoring of Protected Visual Environments) monitors include an aerosol sampler that measures total fine particulate (<2.5 µm diameter) NO_3^- and sulfate (SO_4^-) concentrations over 24 hours every third day. These anions are presumed balanced by NH_4^+ and best correlations between fine particulate N and lichen-based air scores were observed when mean annual averages included N from both sources (IMPROVE 2010; University of California, Davis 1995).

[3] NADP (National Atmospheric Deposition Program); weekly samples of precipitation captured in buckets designed to open during rain and snow events are analyzed for concentrations (mg L^{-1}) and total wet deposition (kg ha^{-1} y^{-1}) of NH_4^+ and NO_3^- and total inorganic N (from NH_4^+ and NO_3^-). Data that meet quality control criteria can be downloaded from the website (NADP 2009).

[4] CMAQ (Community Multiscale Air Quality model). The CMAQ model forecasts wet and dry deposition of 16 primary and secondary N-containing air pollutants: HNO_3, NH_3, NO_2, PAN, NO, RNO_3, PAN2, N_2O_5, HONO, ANH_4I, MA-PAN, ANO_3I, PBZN, ANO_3J, and ANH_4J. Model output accounts for complex interactions among many variables (e.g., landscape, vegetation density, atmospheric chemistry, and climate). Mean annual deposition, based on 1990-1999 emissions data, was calculated on a 36 km grid as described in Porter (2007). For model details, see the online CMAQ science documentation (US EPA 2009). Lichen response was related to the total sum of wet and dry deposition of all 16 air pollutants.

mesic conifer forests of the California Sierras, and dry mixed hardwood-conifer forests of California's Greater Sacramento Valley (Geiser et al. 2010). Input variables required are minimum and maximum mean annual precipitation and a lichen community response threshold (e.g., maximum acceptable percent eutrophs). Mean annual minimum and maximum precipitation within each ecoregion was estimated from Parameter-elevation Regressions on Independent Slopes Model (PRISM) national map of 1971-2000 normals (Daly et al. 2002, PRISM 2010). Expert judgment was used to choose reasonable community response thresholds (Table 4.1). The thresholds are a minimum allowable oligotroph or maximum allowable eutroph percentage of the community composition. Input variables and response threshold justifications are presented in Table 4.1. Basically, forests with hardwood components were assigned a higher response threshold than western Oregon and Washington coniferous forests because the more alkaline bark and richer canopy exudates of hardwoods favor eutrophs. Colder forests were assigned lower response thresholds than warmer forests because native epiphytes are typically oligotrophs adapted to cold- and low-nutrient environments—eutrophs are better adapted to nutrient-concentrated, heat and drought-stressed environments typical of warmer climates. The most problematic areas were the Eastern Forest ecoregion, for which systematically sampled lichen community data exist but are confounded by current or historic levels of N and sulfur (S) deposition and atmospheric concentrations of sulfur dioxide. Because the synergistic effects of multiple pollutants on epiphytic lichens are not well understood, our critical load estimate for eastern forests, based on a western model that accounts for nutrient N deposition alone, are clearly provisional.

4.4 Herbaceous species and shrubs

4.4.1 Response Variables

Responses of herbaceous species to N deposition included changes in species abundances and composition, particularly increases in nitrophilic and invasive species, increases in productivity, and changes in foliar chemistry. In some herbaceous-dominated communities, changes in N cycling and inorganic N leaching below the rooting zone were also used. Typically, the critical loads for these variables were about 10 kg ha^{-1} yr^{-1} above the value estimated from changes in plant composition.

In more impacted ecoregions such as the forested areas of the eastern United States using herbaceous species for critical loads may be problematic, because, as previously stated, N deposition (in combination with S deposition) over the last half century has probably already significantly altered community composition. The critical loads obtained using herbaceous species in these more impacted regions will therefore potentially overestimate the level associated with the initial changes in plant diversity.

4.4.2 Acceptable Studies

We included studies across N deposition gradients and low level N fertilization experiments to evaluate herbaceous species and shrub responses to N deposition. We excluded fertilization studies when single input rates of more than double the current ambient level were used. As noted above, an implicit assumption was made that the primary factor influencing variation in herbaceous species composition in gradient studies was N deposition. We considered studies that included a range of N experimental inputs to be more reliable (##) than those which used single fertilization values or gradient studies.

4.4.3 How the Critical Load Value was Chosen

The critical load value was estimated as the range between the input level at which no response was observed and the lowest level at which an alteration in species abundance or changes in N cycling occurred. In some cases, the input level where a response occurred could be quantitatively assessed by fitting the response data to simple mathematical functions (Bowman et al. 2006). Quantitative changes in species composition for both gradient and N fertilization studies are best determined using point-intercept or frequency estimates. Subtle changes may be missed or misinterpreted using more subjective estimates (e.g., visual estimates of cover). An increase in nitrophilic species cover was used to determine critical loads in tallgrass prairies, Mediterranean shrublands and deserts, and alpine tundra. Changes in community structure, using ordination analysis, was also used in alpine vegetation. In fire-prone western ecosystems, the increased productivity of invasive grasses has promoted increased fire risk, and the productivity threshold under N deposition that promotes fire has been set as the critical load (Rao et al. 2010). Decreases in the diversity of understory herbaceous species were used in eastern forests, again indicating that the initial increase in nitrophilic species has already occurred in these more impacted ecosystems.

Gradient studies were used for only a few ecoregions (e.g., coastal sage scrub). Critical loads were estimated as a range below the deposition rate where significant increases in nitrophilic species occurred. In general, this approach has not been widely used for herbaceous species composition due in large part to the multitude of factors, other than N deposition, that influence herbaceous species composition and chemistry.

Critical loads based on changes in N cycling in an alpine ecosystem were estimated as below the N inputs where significant changes in net N mineralization, nitrification, and inorganic N leaching below the rooting zone were observed

4.5 Forests

4.5.1 Response Variables

The variables considered for forests include changes in percent N in trees and soils, alterations in soil N cycling, which lead to elevated NO_3^- losses, increased susceptibility to secondary stressors (freezing, drought, pests), declines in tree health, changes in tree growth, and increased mortality. We elected to include increases in tree growth as a response, because it is an indication of alteration by N inputs. In Europe, in contrast, growth responses were not considered in setting empirical critical loads for forest ecosystems (Bobbink et al. 2003). In setting the critical load, we only included declines in growth and survivorship and increases in mortality. There is considerable variation in N concentration in tissue by species and site and over time. Thus, setting a threshold value for tissue N concentration as was done for lichens is difficult. Because repeated surveys at the same site are not often available, establishing a value for acceptable percentage increase typically is not feasible.

Changes in species composition, such as those reported for communities of shorter-lived organisms, are infrequently observed for trees. We included some studies that reported decreased tree health and growth and increased mortality, which is observed only rarely. Therefore, even more for trees than other organisms, it is important to understand the mechanisms leading to catastrophic changes to be able to identify early indicators of change.

4.5.2 Acceptable Studies

We included N gradient, fertilization, and long-term studies. Results from long-term, low-level fertilization and gradient studies are considered the most reliable. The additions of N in most forest fertilization studies are much greater than ambient deposition. While we note these high N fertilization studies in our discussion of biomass responses to N deposition, they are typically not relevant for calculating the critical load. Gradient studies have the advantage of demonstrating changes in real time. When a single gradient study was used, the results could not be considered more than fairly reliable, #. When many gradient studies were included (e.g., Aber et al. 2003), the results could be considered reliable, ##.

4.5.3 How the Critical Load Value was Chosen

In some cases (Eastern Temperate and Northern Forest ecoregions) with extensive datasets, we were able to identify an inflection point on the response curve, the point at which the response begins, and thereby identify the critical load with high certainty. We used this approach for NO_3^- losses from forest ecosystems which have been shown to increase above a threshold N deposition (Aber et al. 2003, Wright et al. 2001). In regions with sparser NO_3^- loss data (Northwestern Forested Mountains ecoregion), we were able to identify only the range within which the critical load is most likely to lie. For gradient studies without a clear inflection point, for example, the tree growth and mortality studies in eastern and northern forests, we set the critical load to be greater than the low end of the range of deposition over which the response was observed. For fertilization studies, we identified the critical load as lying in the range between the highest N input for which we observed no response and the lowest N input for which we observed a response.

4.5.4 Extrapolation

For the Tropical and Subtropical Humid Forests ecoregion for which there are no data, we have cautiously extrapolated our critical load values from regions that have similar biota, climate, and soils. Thus we have used, with qualification, the critical loads for the Southeastern Coastal Plain forests as the basis for a provisional estimate for the critical load for the Tropical and Subtropical Humid Forests. We did not extrapolate critical loads for forests to other ecoregions.

4.6 Other Ecosystems

Methods for the Great Plains, North American Deserts, Tropical and Subtropical Humid Forests, Wetlands and Inland surface waters are described in those chapters.

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