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METHODS FOR EVALUATING WETLAND CONDITION #16 Vegetation-Based Indicators of Wetland Nutrient Enrichment





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Prepared jointly by:

The U.S. Environmental Protection Agency Health and Ecological Criteria Division (Office of Science and Technology)

and

Wetlands Division (Office of Wetlands, Oceans, and Watersheds)

NOTICE

The material in this document has been subjected to U.S. Environmental Protection Agency (EPA) technical review and has been approved for publication as an EPA document. The information contained herein is offered to the reader as a review of the "state of the science" concerning wetland bioassessment and nutrient enrichment and is not intended to be prescriptive guidance or firm advice. Mention of trade names, products or services does not convey, and should not be interpreted as conveying official EPA approval, endorsement, or recommendation.

APPROPRIATE CITATION

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http://www.epa.gov/ost/standards

http://www.epa.gov/owow/wetlands/bawwg

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Foreword

In 1999, the U.S. Environmental Protection Agency (EPA) began work on this series of reports entitled *Methods for Evaluating Wetland Condition*. The purpose of these reports is to help States and Tribes develop methods to evaluate (1) the overall ecological condition of wetlands using biological assessments and (2) nutrient enrichment of wetlands, which is one of the primary stressors damaging wetlands in many parts of the country. This information is intended to serve as a starting point for States and Tribes to eventually establish biological and nutrient water quality criteria specifically refined for wetland waterbodies.

This purpose was to be accomplished by providing a series of "state of the science" modules concerning wetland bioassessment as well as the nutrient enrichment of wetlands. The individual module format was used instead of one large publication to facilitate the addition of other reports as wetland science progresses and wetlands are further incorporated into water quality programs. Also, this modular approach allows EPA to revise reports without having to reprint them all. A list of the inaugural set of 20 modules can be found at the end of this section.

This series of reports is the product of a collaborative effort between EPA's Health and Ecological Criteria Division of the Office of Science and Technology (OST) and the Wetlands Division of the Office of Wetlands, Oceans and Watersheds (OWOW). The reports were initiated with the support and oversight of Thomas J. Danielson (OWOW), Amanda K. Parker and Susan K. Jackson (OST), and seen to completion by Douglas G. Hoskins (OWOW) and Ifeyinwa F. Davis (OST). EPA relied heavily on the input, recommendations, and energy of several panels of experts, which unfortunately have too many members to list individually:

- Biological Assessment of Wetlands Workgroup
- Wetlands Nutrient Criteria Workgroup

More information about biological and nutrient criteria is available at the following EPA website:

http://www.epa.gov/ost/standards

More information about wetland biological assessments is available at the following EPA website:

http://www.epa.gov/owow/wetlands/bawwg

LIST OF "METHODS FOR EVALUATING WETLAND CONDITION" MODULES

Module #	Module Title
1	INTRODUCTION TO WETLAND BIOLOGICAL ASSESSMENT
2	INTRODUCTION TO WETLAND NUTRIENT ASSESSMENT
3	THE STATE OF WETLAND SCIENCE
4	Study Design for Monitoring Wetlands
5	Administrative Framework for the Implementation of a
	Wetland Bioassessment Program
6	DEVELOPING METRICS AND INDEXES OF BIOLOGICAL INTEGRITY
7	WETLANDS CLASSIFICATION
8	Volunteers and Wetland Biomonitoring
9	Developing an Invertebrate Index of Biological
	INTEGRITY FOR WETLANDS
10	Using Vegetation to Assess Environmental Conditions
	IN WETLANDS
11	Using Algae to Assess Environmental Conditions in
	WETLANDS
12	Using amphibians in Bioassessments of Wetlands
13	BIOLOGICAL ASSESSMENT METHODS FOR BIRDS
14	WETLAND BIOASSESSMENT CASE STUDIES
15	BIOASSESSMENT METHODS FOR FISH
16	VEGETATION-BASED INDICATORS OF WETLAND NUTRIENT
	ENRICHMENT
17	Land-Use Characterization for Nutrient and Sediment
	RISK ASSESSMENT
18	BIOGEOCHEMICAL INDICATORS
19	NUTRIENT LOAD ESTIMATION
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SUMMARY

Z egetation-based attributes of wetland function (e.g., energy flow, nutrient cycling) and structure (species composition) that respond to nutrient enrichment and eutrophication are presented below. Attributes consist of Level I and Level II indicators that respond quickly to nutrient enrichment and are relatively easy to use. Level I indicators consist of remotely sensed data to assess change in wetland plant communities over time as well as field measurements of stem height and leaf C:N:P ratios. Level I indicators are recommended for coarse assessment of individual wetlands or large-scale surveys of many Level II indicators include wetlands. aboveground biomass/litterfall, standing dead C:N:P, nutrient resorption efficiency and proficiency, nutrient use efficiency, and nutrient-tolerant and -intolerant species. These indicators are used for more detailed assessment of wetland eutrophication. Their sound application requires that similar sample collection protocols be used for both "targeted" (potentially eutrophic) and reference (unenriched) wetlands.

Observational and experimental studies confirm the reliability of vegetation-based indicators for identifying eutrophication in nutrient-enriched and unenriched areas of the Florida Everglades, salt marshes, and wet sedge tundra. Until the indicators are tested elsewhere, however, they should be applied cautiously to assessments of eutrophication in other types of wetland and in different geographic regions.

PURPOSE

The purpose of this module is to identify veg etation-based indicators that can be used by wetland regulatory and natural resource managers to determine the nutrient status (eutrophic or unenriched) of freshwater and estuarine wetlands.

INTRODUCTION

W etlands improve surface water quality by intercepting sediment, nutrients, and other pollutants transported from terrestrial areas and upstream aquatic ecosystems (Johnston 1991, Mitsch and Gosselink 1993). Wetlands become sinks for nitrogen (N) by sequestering it in accumulating soil organic matter (Craft 1997) and by microbially converting nitrate (NO_3^{-1}) to atmospheric N₂ (denitrification) (Groffman 1994). Wetlands serve as sinks for phosphorus (P) by trapping sediments; by sorption to iron (Fe), aluminum (Al), and calcium (Ca) minerals; and by plant uptake (Craft 1997). The ability of wetlands to remove nutrients has led to its widespread use, in both natural and artificial forms, to remove N and P from secondarily treated wastewater, septic effluent, and nutrient-enriched agricultural drainage (Johnston et al. 1991, Craft and Richardson 1993, Kadlec and Knight 1996). When natural wetlands receive excessive nutrient loadings, ecosystem processes, such as plant productivity and nutrient cycling, are altered in measurable ways. The structure of the plant community also may change as slower growing native species are replaced by faster growing species that take advantage of high nutrient levels to increase growth (Davis 1991).

The threshold where significant alteration in wetland function and structure occurs is referred to as the "assimilative capacity" of the system. When the assimilative capacity of a wetland is exceeded, the ecosystem responds by increasing nutrient uptake that translates into increased growth. Sometimes the result is a shift in plant species composition, as natives are displaced by aggressive interlopers like cattail (*Typha*). This phenomenon is known as cultural eutrophication and is caused by excessive nutrient loadings from anthropogenic sources. Because N and P are the primary nutrients limiting productivity in wetlands (Schlesinger 1991, Vitousek and

Howarth 1991, Bridgham et al. 1996), these nutrients usually are responsible for changes in ecosystem function and structure that occur when wetland assimilative capacity is exceeded (Carpenter et al. 1998).

Wetland vegetation responds to nutrient additions by increased storage of N and P in plant tissue and by increased net primary production (NPP) (Craft et al. 1995, Bridgham et al. 1996). Increased NPP and nutrient storage in turn affect ecosystem processes including decomposition (Valiela et al. 1982, Davis 1991, Rybczyk et al. 1996), accumulation of soil organic matter, and organic carbon export (Craft and Richardson 1993, 1998, Morris and Bradley 1999). Over time, plant species composition may shift as native species decline and are replaced by species that take advantage of high nutrient levels to increase growth (Craft et al. 1995). Nutrient enrichment often results in replacement of uncommon or rare species by species tolerant of high nutrient loadings (e.g., Typha, Phragmites) (Davis 1991, Chambers et al. 1999, Galatowitsch et al. 1999). Such changes in community composition and ecosystem processes compromise wetland ecological integrity by altering energy flow, nutrient cycling, and niche/habitat characteristics that in turn affect wetland fauna assemblages.

This chapter describes vegetation-based indicators that can be used to determine whether a wetland's ecological integrity has been impaired by nutrient enrichment and eutrophication. Indicators are described for structural and functional responses to both low and high nutrient loadings. Functional indicators include leaf N and P content and metrics of NPP (biomass production, stem height). Structural indicators consist of the presence/absence of "sentinel" species that reflect ambient (low nutrients) and impaired (high nutrients) nutrient loading regimes. Methods for sampling vegetation and analytical techniques to assess the degree of nutrient enrichment also are described.

VEGETATION-BASED INDICATORS OF NUTRIENT ENRICHMENT

N Jutrient enrichment affects both structural and functional attributes of wetlands. Structural attributes include characteristics of the community, or of individual species, whereas functional attributes relate to energy flow and nutrient cycling. Changes in wetland function that occur in response to nutrient enrichment include increased N and P uptake, NPP, and decomposition. Changes in structure occur through shifts in plant species composition, including replacement of nutrient-intolerant species with those adapted to high nutrient conditions. In this module, functional indicators are emphasized because energy flow and nutrient cycling processes respond quickly and dramatically when nutrient loadings are increased.

FUNCTIONAL INDICATORS

When nutrients are limiting, wetland vegetation responds to nutrient additions by incorporating N and P into growing or "green" tissue and increasing NPP (Shaver and Melillo 1984, Craft et al. 1995, Koerselman and Meuleman 1996, Shaver et al. 1998). Changes in nutrient uptake and NPP affect wetland energy and nutrient cycles by altering rates of uptake, storage, and release of C, N, and P.

Net primary productivity

Net primary productivity is the amount of carbon fixed during photosynthesis that is incorporated into new leaves, stems, and roots. NPP is often expressed as amount of biomass produced per m² of wetland surface per year ($g/m^2/yr$). Most

techniques to measure NPP focus on production of aboveground biomass and discount root production that sometimes accounts for half or more of the NPP. The simplest way to measure aboveground biomass is by harvesting all of the standing material at the end of the growing season (Broome et al. 1986). This method is useful for measuring NPP of herbaceous emergent vegetation, especially in temperate climates where there is a distinct growing season. However, measurements of aboveground or "standing crop" biomass typically underestimate NPP because they do not include biomass losses to herbivory and stem mortality during the growing season. Nondestructive methods such as tagged stems or the use of external markers like wire are used to measure NPP of Sphagnum (Clymo and Hayward 1982) and coastal Louisiana marsh plants (Hopkinson et al. 1980). These methods account for stem mortality and herbivory, and provide a truer estimate of NPP than the harvest method. However, they are much more labor-intensive, and timeconsuming, and are not recommended for wetland eutrophication assessment. Enhanced biomass production is reflected by increased height and, sometimes, stem density of herbaceous emergent vegetation (Broome et al 1983). Increased stem density, however, may reflect other factors like vigorous clonal growth, so it is not recommended as an indicator of nutrient enrichment.

Woody vegetation is not as good an indicator of enrichment as is herbaceous vegetation. Woody plants grow slowly and have a longer life cycle than herbaceous plants, resulting in a slower response to nutrient loading. In wetland dominated by trees with little herbaceous vegetation, leaf litterfall is a common means to estimate NPP (Chapman 1986). Measurements of litterfall involve the periodic (usually monthly) collection of leaf litter that collects in littertraps placed on or above the forest floor. Like the harvest method, litterfall is an index of NPP because it estimates the portion of NPP that goes into producing photosynthetic tissue. One drawback, however, is that the litterfall method is labor-intensive and time-consuming. But, for wetlands where herbaceous vegetation is unimportant, litterfall is the best method to measure NPP.

Biogeochemical cycling

Indicators of biogeochemical cycling describe the uptake, storage, and release of N and P in plant tissue. Nutrient enrichment of wetlands leads to increased uptake and storage of N and/ or P, depending on the causative nutrient (Verhoeven and Schmitz 1991, Shaver et al. 1998). In wetlands where P is limiting, leaf tissue P is the first indicator to respond to nutrient enrichment (Craft et al. 1995). Increased P uptake by plants is known as "luxury uptake" because P is stored in vacuoles and used later (Davis 1991). Like P, leaf tissue N increases in response to N enrichment (Brinson et al. 1984, Shaver et al. 1998). However, most N is used directly to support photosynthesis and growth of new tissue, so luxury uptake of N is not always observed (Verhoeven and Schmitz 1991).

The ratio of carbon to nitrogen (C:N) in aboveground biomass or leaves can be used to determine whether a wetland is N-limited or whether there is excess N in the system. Under conditions of N enrichment, plants assimilate more N, increasing leaf N and decreasing C:N (Shaver and Melillo 1984, Shaver et al. 1998). Likewise, P enrichment results in increased leaf P and decreased C:P (Craft et al. 1995). Application of C:N and C:P ratios requires that baseline measurements are made using vegetation collected from unenriched areas or from the same area prior to eutrophication.

Leaf N:P also has been used to determine whether a wetland is N-limited or P-limited (Koerselman and Meulemen 1996, Verhoeven et al. 1996). It has been hypothesized that N:P < 15 (weight:weight basis) indicates N limitation whereas N:P>15 indicates P limitation (Verhoeven et al. 1996). Assuming that this hypothesis is valid, this information is useful for determining whether a wetland is at risk for either N or P enrichment. For example, wetlands with vegetation N:P<15 may be susceptible to N enrichment whereas wetlands with vegetation N:P>15 may be susceptible to P limitation. Sometimes N:P ratios are presented on a mole:mole basis. In this case, N:P <33 indicates N limitation whereas N:P>33 suggests P limitation. Development of techniques to identify N versus P limitation is important because, for a given wetland, the nutrient that limits ecosystem productivity usually is the cause of eutrophication.

Three useful measures of nutrient availability and eutrophication are nutrient resorption efficiency, nutrient resorption proficiency, and nutrient use efficiency (Shaver and Melillo 1984, Killingbeck 1996). Nutrient resorption efficiency is a measure of nutrient conservation and limitation. Under low nutrient conditions, plants resorb and translocate nutrients from senescing tissue and store them in belowground tissue to be used later (Aerts et al. 1999). Vegetation growing in nutrient-poor wetlands translocate large quantities of nutrients to belowground tissue and, thus, possess high nutrient resorption efficiency. Plants growing in nutrient-enriched wetlands often possess low nutrient resorption efficiency because of high nutrient availability in soil and water. Nutrient resorption efficiency (RE) is defined as:

$$RE = \frac{N \text{ or } P(g/m^2) \text{ in green biomass}}{N \text{ or } P(g/m^2) \text{ in standing}} \times 100\%$$

where biomass is aboveground clipped (har-vested) material.

Resorption efficiency also may be calculated using the concentration of N or P (mg) per individual leaf (Aerts et al. 1999) or per cm² leaf material (Feller et al. 1999).

Nitrogen and P in senesced or standing dead leaves also are used as measures of nutrient resorption proficiency. Nutrient resorption proficiency is defined as the absolute levels to which nutrients are reduced in senesced leaves (Killingbeck 1996). Resorption is highly proficient in plants that reduce N and P concentrations below 0.7% and 0.05%, respectively. It is thought that high resorption proficiency is an evolutionary adaptation that enables plants to conserve nutrients in infertile environments (Killingbeck 1996).

Nutrient use efficiency (NUE) is a measure of the effectiveness by which plants use nutrients to produce biomass. High NUE corresponds to high rates of nutrient resorption because large amounts of biomass are produced with little loss of nutrients in litterfall (Vitousek 1982). Nutrient-poor wetlands often possess lower litter N and P concentrations, resulting in a higher NUE than in nutrient-rich wetlands (see Table 2). Nutrient use efficiency is defined as:

Aboveground biomass production (e.g., litterfall, standing dead biomass) NUE =

Nutrient (N or P) in litterfall, standing dead biomass

where litterfall/biomass and nutrients are expressed in g/m^2 (Vitousek 1982).

A simpler means to measure NUE is by calculating the inverse of the concentration of N or P in standing dead biomass/litterfall (Aerts et al. 1999). The major advantage of this method over the standard NUE method is that litterfall need not be measured, only tissue N and/or P content. Nutrient use efficiency varies widely among different growth forms such as grasses, conifers, and deciduous trees and shrubs. For example, conifers typically have much higher N and P use efficiency than deciduous and graminoid wetland plants (Aerts et al. 1999). For this reason, it is important to compare NUE, RE, and the resorption proficiency of similar growth forms and species collected from nutrient-enriched and unenriched wetlands to assess the vegetation response to eutrophication.

STRUCTURAL INDICATORS

Structural indicators of nutrient enrichment consist of community-level attributes like the presence or absence of specific species. Cattail (*Typha*), for example, encroaches on and colonizes areas that have undergone soil disturbance, nutrient enrichment, and hydrology alteration (Apfelbaum 1985, Urban et al. 1993). Other species are common in nutrient-poor wetlands, but decline or disappear during eutrophication as they are overcome displaced (Davis 1989, Craft et al. 1995, Jensen et al. 1995). Attributes that describe vegetation structure, like biomass and stem height, also are useful indicators of enrichment.

Structural indicators of nutrient enrichment include biomass, stem height (discussed earlier), dramatic/widespread change in plant community composition over time, and the presence or absence of species adapted to either nutrient-enriched (nutrient-tolerant) or unenriched (nutrient-intolerant) conditions. Biomass and stem height also provide an index of NPP, which increases in response to enrichment. Other possible structural indicators include species dominance, richness, and the presence of rare and invasive (nonnative) species.

Structural indicators may be less reliable than functional indicators for wetland eutrophication assessments because plants respond to environmental fac-

tors other than nutrients. Light, moisture/waterlogging, acidity, and other stressors (e.g., salinity, sulfides, fire) affect plant community composition (Smith 1996). In addition, different plant species possess variable life history traits, such as seed production, dispersal, viability, and germination, that determine their distribution across the landscape (van der Valk and Davis 1978). Environmental factors and life history traits interact to regulate the geographic distribution of plant species. Structural indicators, described above, also can be used to assess the overall biological integrity of wetlands. Module 10: Using Vegetation to Assess Environmental Conditions in Wetlands provides a detailed overview of using vegetation to assess biological integrity (Fenessy in press).

Anthropogenic disturbances to wetlands often are manifested in a dramatic and widespread change in plant species composition over time. Aerial and satellite photography can be used to detect coarse changes in wetland plant communities in response to eutrophication (Jensen et al. 1987, 1995). Remote sensing techniques can detect changes in the aerial extent of wetlands, the percent cover of vegetation, as well as the replacement of one plant community by another. Remote sensing requires field verification, however, to calibrate plant community types with patterns discerned from aerial and satellite images. (See Module 17: Land-Use Characterization for Nutrient and Sediment Risk Assessment. for further information on this topic.) Field-based measurements of NPP and biogeochemical indicators also are needed to verify whether eutrophication and not some other type of disturbance (e.g., hydrologic alteration) is the causative agent.

During the eutrophication process, large-scale shifts in plant species composition occur in response to the addition of the limiting nutrient. For example, cattail encroaches on and eventually can become the dominant species in areas of the Everglades that

receive large loadings of P, the primary limiting nutrient (Davis 1991). In wetlands where surface water is present, duckweed (Lemna sp.) often increases in density and coverage in response to increased nutrients (Portielje and Roijackers 1995, Janse 1998, Vaithiyanathan and Richardson 1999). Another species that may invade in response to increased nutrients is Phragmites (Chambers et al. 1999, Galatowitsch et al. 1999). It is important to be aware that some species invade in response to other anthropogenic alterations like changes in hydroperiod (e.g., Typha sp., Phragmites australis, Phalaris arundinacea) and salinity (e.g., Phragmites, Typha angustifolia) and soil disturbance (Typha sp., Lythrum salicaria, Phragmites, Phalaris) (Apfelbaum 1985, Urban et al. 1993, Chambers et al. 1999, Galatowitsch et al. 1999). Thus, when using species-specific indicators, it is important to identify anthropogenic disturbances in and around the wetland to ensure that changes in plant species composition are the result of nutrient enrichment and not some other type of disturbance.

In contrast to nutrient-tolerant species, some species are adapted to low nutrient or oligotrophic conditions. In the Everglades, emergent (sawgrass, Cladium jamaicense) and floating aquatic (bladderwort, Utricularia sp.) vegetation dominate unenriched areas but are replaced by cattail and other species in eutrophic areas (Davis 1989, Urban et al. 1993). The disappearance of submerged aquatic vegetation (SAV) also may be a useful indicator of nutrient enrichment. Over the past few decades, SAV declined dramatically in many estuaries of the eastern United States. (Orth and Moore 1983). The decline of SAV has been linked to light attenuation caused by eutrophication and sedimentation (Dennison et al. 1993). It should be noted that species identified as nutrient intolerant may be limited to specific wetland types and geographic regions.

A national database of wetland plant sensitivities to nutrient enrichment and hydrologic alteration is being produced for USEPA by Paul Adamus of Oregon State University (Adamus and Gonyaw 2000). The database, which is to include both experimental and observational studies, assesses the responses of various species to eutrophication and alteration of wetland hydrology. The database may serve as a guide to identify wetland species that are indicators of nutrient enrichment.

METHODS FOR Assessing Nutrient Enrichment

ssessing wetland to detect nutrient enrich-A ment is different from assessing it for biological integrity. The goals of an Index of Biological Integrity (IBI) are to assess the health of the biotic or living components of the ecosystem, using metrics such as species richness, and to relate biotic health to anthropogenic stressors, such as land clearing, drainage, and runoff, that affect the community undergoing study. The purpose of this module, in contrast, is to identify vegetation-based indicators of a specific stressoreutrophication-whose effects are manifested as altered ecological structure and function, especially energy flow and nutrient cycles. Despite their differences, however, both the IBI and the assessment for nutrient enrichment share a similar framework for achieving their respective goals. For both types of assessments, it is important to follow the procedures outlined in Module 6: Developing Metrics and IBIs. Like IBIs, assessments for nutrient enrichment should (1) define clear objectives, (2) classify wetlands into regional classes, (3) carefully select reference sites and sample sites, and (4) collect information on wetland characteristics (e.g., hydrology, wetland vegetation, soils) and surrounding land use. In particular, landscape level and local disturbances that contribute to nutrient enrichment should be identified.

Wetland assessment for eutrophication requires collection of remotely sensed and field data. Aerial and satellite photography are used to document changes in aerial coverage and percent cover of wetland vegetation as well as coarse changes in plant community composition over time. Remotely sensed data are obtained from a variety of government agencies, including NASA; USDA-NRCS; State highway departments; and local tax, planning, and zoning offices. Information for obtaining these data are found in Module 17: Land-Use Characterization for Nutrient and Sediment Risk Assessment.

Field data consist of Level I and Level II indicators of nutrient enrichment that are based on attributes of wetland ecosystem function and structure.

- Level I indicators (stem height, plant tissue N and P) are relatively easy to measure.
- Level II indicators (aboveground biomass, nutrient use efficiency, presence/absence of nutrient-tolerant and intolerant species) require greater effort, but better characterize the response of specific structural and functional attributes to nutrient enrichment.

FIELD SAMPLING

In addition to remotely sensed data, field and laboratory measurements are needed to assess changes in wetland structure and function that occur in response to nutrient enrichment. The best way to document change in structure and function is by monitoring the site over time, before and during the eutrophication process. This opportunity rarely occurs because, in most cases, wetland eutrophication began decades ago following widespread application of inorganic fertilizers.

A widely used approach to assess anthropogenic impacts is to compare the ecological integrity of potentially enriched wetlands with unenriched "reference" wetlands. For example, unenriched areas of the southern Everglades have been used as a reference in documenting the effects of enrichment in areas of the northern Everglades that receive agricultural drainage (Davis 1989, 1991, Craft and Richardson 1993, 1998, Reddy et al. 1993, Qualls and Richardson 1995). The ideal reference wetland is one that is relatively undisturbed and possesses the same abiotic template, except for nutrients, as the enriched wetland. When using reference wetlands, a key assumption is that the reference site contains the same assemblage of biota that enriched wetland contained prior to eutrophication. Usually, reference wetlands are selected from the same watershed or from a watershed nearby so that both enriched and reference wetlands possess the same abiotic template of climate, geomorphology, geology, hydrology, and soil type.

It is nearly impossible to find wetlands that have been unaffected by at least some human activity. Wetlands that are minimally disturbed usually represent the best approximation. Sampling more than one reference wetland is highly recommended to fully characterize the natural variability among a particular wetland type and to minimize the effects of human disturbance inherent in one or more reference sites.

Protocols for field sampling should be designed to capture the spatial and temporal variability inherent in both candidate and reference wetlands. It is critical that the same experimental design, frequency of sampling, field measurements, and laboratory methods be used for both nutrient-enriched and reference wetlands. Because hydrology exerts a controlling influence on wetland structure and function, a stratified sampling approach is needed to encompass the spatial variation in inundation patterns. Sampling is stratified into deep-, mid-, and shallow-water zones or from more to less frequently flooded areas (Figures 1 and 2) (see also Module 6: Developing Metrics and IBIs). Using hydrology to stratify sampling captures the distinct patterns of zonation of plant and animal communities that often are observed in wetlands. Deep-water zones, which are in contact with water (and nutrients) longer than shallow-water zones, may respond to enrichment relatively quickly compared with higher elevations that are flooded less frequently.

In some wetlands, the presence of inundation or soil saturation occurs only for short periods during the growing season. When sampling these wetlands, it may be necessary to design a sampling plan that encompasses the temporal variability in inundation that affects seasonal changes in plant and animal communities.

Selection of sampling locations depends on the size and habitat complexity of the wetland. For small-sized wetlands or surveys of many wetlands, sampling is stratified according to habitat complexity or water-level depth. In depressional or shoreline wetlands, samples and measurements are taken near the center (deepwater), middle, and edge (shallow) of the wetland (see Figure 2). In floodplain wetlands, sampling is stratified based on habitat complexity with samples collected from the levee, oxbow, and terrace habitats (see Figure 1). Large wetlands or comprehensive studies require greater sampling effort. Comprehensive assessments require replicate sampling points within a given location or habitat and repeated measurements at a given location to accurately assess changes in nutrient indicators over time.

Collection of vegetation for nutrient and carbon analysis requires careful selection of leaf samples. When comparing nutrient-enriched and reference wetlands, it is important that the same species be sampled in each wetland because different species possess different amounts of N and P in their leaves (Craft et al. 1995, Shaver et al. 1998, Aerts et al. 1999). Herbaceous species preferably are sampled (unless it is a forested wetland) because they grow faster and respond to enrichment faster than woody species. The two or three dominant species based on percent cover or biomass are sampled. It also is important to collect similar-aged green leaves from each site, as young leaves usually have higher N and P content than older leaves (Schlesinger 1991). In the case of herbaceous vegetation, similar-aged leaves are selected by clipping leaves from nodes of a similar distance below the terminal bud. From woody vegetation, green leaves are selected similarly by sampling a fixed number of nodes (or branches) below the terminal bud. Replicate leaf samples are collected from several individual plants to encompass the variability in leaf N and P within populations.

A "flow" diagram describing steps for field sampling of wetland vegetation is provided in Figure 3.

ANALYTICAL METHODS

Different methods are required to measure NPP in herbaceous as opposed to woody vegetation. For herbaceous vegetation, stem height and biomass are used to measure NPP. Biomass is determined by end-of-season harvest of aboveground plant material in small (0.25 m²) plots (Broome et al. 1986). The stem height of individuals of dominant species is measured in each plot. The height of the 5 to 10 tallest stems in each plot has been shown to be a reliable indicator of NPP (Broome

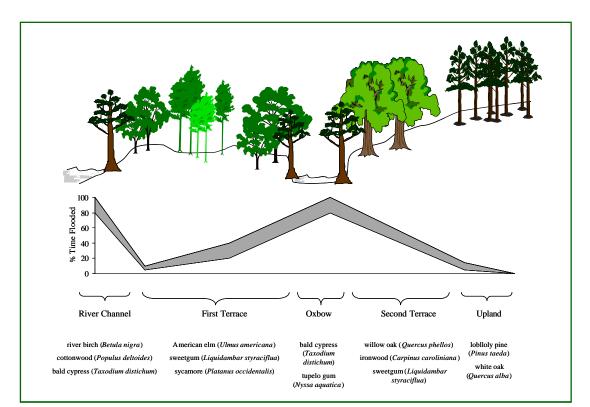


FIGURE 1: DISTRIBUTION OF PLANT SPECIES AND HYDROPERIOD ACROSS A SOUTHEASTERN BOTTOMLAND FORESTED WETLAND.

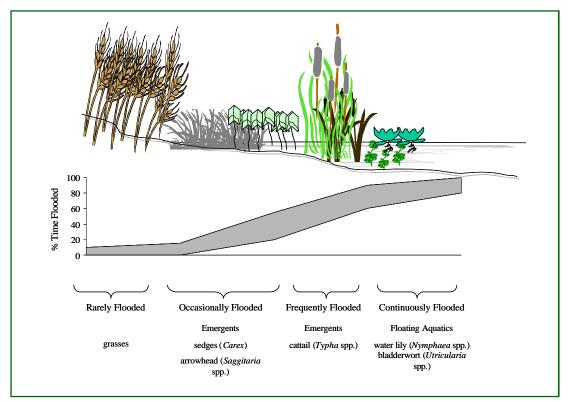


FIGURE 2: DISTRIBUTION OF PLANT SPECIES AND HYDROPERIOD ACROSS A FRESHWATER MARSH WETLAND.

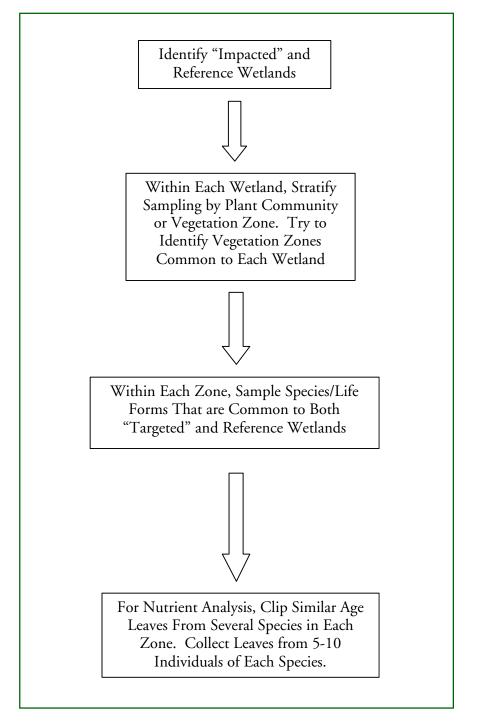


FIGURE 3: PROTOCOL FOR SAMPLING VEGETATION FOR WETLAND EUTROPHICATION ASSESSMENTS.

et al. 1986) and one that saves the time it takes to measure the height of all stems in a plot. Aboveground biomass is clipped at the end of the growing season, in late summer or fall. If vegetation is dense, 0.25 m^2 plots are sufficient for clipping. Clipped material is separated into live (biomass) versus dead material then dried at 70°C to a constant weight. For stem height and biomass sampling, 5 to 10 plots per vegetation zone are collected.

For woody vegetation, litterfall is the best technique for measuring NPP. Litterfall is measured by collecting leaf litter that falls into 0.25 m² screen or mesh traps placed on the wetland surface (Chapman 1986). Collections are made periodically (every 1-2 mos.) throughout the year, although collection during peak litterfall season (Sept.-Dec.) may be adequate for some assessments.

Leaves are collected and analyzed for N, P, and organic C. Leaf analyses are performed on samples that are dried at 70°C. Nitrogen and organic C are measured by dry combustion using a CHN analyzer. Phosphorus is measured by spectrophotometry in acid $(H_2SO_4-H_2O_2)$ digests (Allen et al. 1986). Many land-grant universities, state agricultural testing laboratories, and environmental consulting laboratories perform these analyses. Contact your local USDA office or land-grant agricultural extension office for information on laboratories that perform plant tissue nutrient analyses.

Nutrient resorption efficiency, resorption proficiency, nutrient use efficiency, and C:N:P are calculated from the C, N, and P concentrations measured previously. Resorption proficiency and RE require that N and P are analyzed for both senesced and green tissue (Killingbeck 1996, Aerts et al. 1999). Nutrient use efficiency requires measurements of productivity (litterfall, aboveground biomass) and leaf N and P (Vitousek 1982). For emergent vegetation, community-level indicators (nutrient-tolerant and intolerant species) are measured in larger plots $(2-10 \text{ m}^2)$, or by estimating percent cover of each species using plot or plotless sampling techniques (Mueller-Dombois and Ellenberg 1974). The use of community-level indicators with woody vegetation requires larger plots (0.1-1 ha) or longer transects (Mueller-Dombois and Ellenberg 1974).

MINIMUM MONITORING REQUIREMENTS

Minimum monitoring requirements to assess nutrient enrichment of wetlands consist of (1) aerial/ satellite photography of the wetland and (2) fieldbased measurements of Level I indicators of enrichment. Level I indicators describe attributes of wetland structure (stem height) and function (stem height, leaf C, N, P and C:N:P ratios) that are relatively easy to measure (Table 1).

Remotely sensed data are used to assess coarse changes in wetland communities over time. Field measurements such as stem height and leaf N and P are useful because they respond to nutrient enrichment relatively quickly (Craft et al. 1995, Chiang et al. 2000). Herbaceous vegetation is a better indicator of nutrient enrichment than woody vegetation because herbaceous plants complete their life cycle in less time and, thus, respond more quickly to enrichment.

CASE STUDIES

Case studies using the Florida Everglades, Atlantic coast estuarine salt marshes, and Alaskan wet sedge tundra are presented below to describe the response of vegetation-based indicators to nutrient enrichment. The response of Everglades plant communities to N and P additions is an example of phosphorus limitation and eutrophication. The response of the estuarine salt marshes,

TABLE 1: LEVEL I AND LEVEL II INDICATORS OF NUTRIENT ENRICHMENT IN WETLANDS

MEASUREMENT	Ease of Use	Метнор				
Level I						
Functional Indicators						
Stem height (E) ^{1,2}	Clip plots					
Leaf C and N, C:N	Moderate	CHN analyzer				
Leaf P, C:P, N:P	Moderate	Acid digestion & spectrophotometer				
Structural Indicators						
Coarse change in wetland plant communities over time	Easy	Aerial & satellite photography				
Level II	-					
Functional Indicators	_					
Aboveground biomass (E)	Moderate	Clip plots				
Standing dead biomass (E)	Moderate	Clip plots				
Litterfall (W) ¹	Difficult	Litter traps				
Senesced leaf C, N, C:N ³	Moderate	CHN analyzer				
Senesced leaf P, C:P, N:P ³	Moderate	Acid digestion & spectrophotometer				
N & P resorption efficiency	—	Calculated				
Nutrient use efficiency	—	Calculated				
Structural Indicators	Structural Indicators					
# of nutrient tolerant species ⁴	Moderate	Plot sampling				
% of nutrient tolerant species ⁴	Moderate	Plot sampling				
# of nutrient intolerant species ⁴	Moderate	Plot sampling				
% of nutrient intolerant species ⁴	Moderate	Plot sampling				

 1 E = emergent vegetation, W = woody vegetation.

² Structural indicator also.

³ Resorption proficiency.

⁴ Reliable nutrient-tolerant and -intolerant species for most wetlands and geographic regions have not been identified yet.

Note: Level I indicators represent the minimum requirements for assessing enrichment.

which are N-limited, is an example of wetland response to N enrichment. The response of wet sedge tundra to nutrient enrichment is an example of colimitation by N and P. In the tundra wetland, vegetation-based indicators of nutrient enrichment show a response to both N and P additions.

Everglades

During the past 10 years, the Florida Everglades has been the "poster child" for wetland eutrophication. Numerous studies documenting the effects of N- and P-enriched agricultural drainage on Everglades community structure and ecosystem processes have been published (Davis 1989, 1991, Craft and Richardson 1993a, 1998, Craft et al. 1995, Reddy et al. 1993, Urban et al. 1993). Ecological changes attributed to nutrient enrichment include increased NPP, tissue P uptake, decomposition, peat accretion, and nutrient accumulation as well as cattail encroachment into sawgrass and slough communities (Craft and Richardson 1998, Qualls and Richardson 2000). Functional indicators of enrichment including aboveground biomass, stem height, leaf N (standing dead only), and leaf P were higher in eutrophic areas compared with unenriched areas (Table 2). Stem density did not differ between eutrophic (40/m²) and unenriched sites (38/m²) (Miao and Sklar 1999).

Community-level indicators also responded to increased nutrient loadings. Nutrient- tolerant species including cattail, duckweed (*Lemna*), and other species were abundant in eutrophic areas but infrequent in unenriched areas (Table 2), (Craft and Richardson 1997, Vaithiyanathan and Richardson 1999). Nutrient-intolerant species (e.g., *Utricularia* spp. and other species) were abundant in unenriched areas but absent from eutrophic areas (Table 2) (Vaithiyanathan and Richardson 1999).

The findings presented above were based on observational data collected from areas of the northern Everglades that receive enormous amounts of water and nutrients, both N and P. From among these data, it was difficult to separate the effects of nutrients from the hydroperiod, and to determine which responses were due to N versus P. Thus the causative agent of the observed differences was not pinpointed.

In 1990, a field experiment was initiated to investigate the effects of N versus P on native Everglades plant communities (Craft et al. 1995, Chiang et al. 2000). The experiment applied controlled amounts of N, P, and N+P to plots in an area of the Everglades unaffected by agricultural water and nutrient loadings. During the first year of nutrient additions, it became apparent that P, not N, was the limiting nutrient and, thus, was responsible for the changes in wetland structure and function observed in eutrophic areas. After 2 years of P additions, many functional indicators responded to increased P, including increased aboveground biomass, standing dead material, stem height, and leaf P (Table 3). Leaf C:P, N:P, resorption efficiency, and NUE (P) decreased in response to P additions (Table 3). Sentinel species that reflected low nutrient regimes also responded to P. Utricularia, a floating aquatic plant, declined in response to P and eventually was replaced by the macroalgae, Chara. During the 4-year period, no change in species richness or cattail/duckweed encroachment was observed, although other emergents like leather fern (Acrostichum danaeifolium) increased in response to P (Table 3). More leaf P and less Utricularia indicated incipient P enrichment during the first year of P additions. The response of aboveground biomass to P was not statistically detectable until the end of the second growing season. Under unenriched conditions, sawgrass was highly proficient at resorbing P based on standing dead P (70 $\mu g/g$) concentrations that were less than 500 $\mu g/g P$ as suggested by Killingbeck (1996). There was no response of vegetation to N additions (data not shown), indicating that P, not N, limits productivity and leads to eutrophication in this wetland system.

TABLE 2: COMPARISON OF VEGETATION-BASED INDICATORS OF PHOSPHORUS (P) ENRICHMENT IN UNENRICHED AND EUTROPHIC SAWGRASS, CLADIUM JAMAICENSE, COMMUNITIES OF THE FLORIDA EVERGLADES

Indicator	Unenriched	Eutrophic	
Functional Indicators			
Stem height (cm) ¹	160	205	
Aboveground biomass (g/m ²) ¹	976	1958	
Stem density (number/m ²) ¹	38	40	
Leaf N (%) ¹	0.70	0.70	
Leaf P $(\mu g/g)^1$	250	650	
Leaf N:P (wt:wt) ¹	28	11	
Standing dead N (%) ²	0.40	0.56	
Standing dead P $(\mu g/g)^2$	80	375	
Standing dead N:P (wt:wt) ²	38	15	
Structural Indicators			
Course change in wetland plant communities over time ³	No change	Decreased sawgrass, increased cattai	
Nutrient-intolerant species			
Utricularia spp. ⁴	Abundant	Absent	
Nutrient-tolerant species			
Typha ^{4,5}	Infrequent	Abundant	
Lemna ⁴	Absent	Abundant	
Acrostichum danaeifolium ⁴	Infrequent	Common	

¹ From Miao and Sklar (1999)

² From Davis (1991)

³ Jensen et al. (1995)

⁴ From Vaithiyanathan and Richardson (1999)

⁵ From Craft and Richardson (1997)

Note: Numerical values in bold reflect statistically significant differences (p>0.05) between unenriched and eutrophic conditions.

TABLE 3: LEVEL I AND LEVEL II INDICATORS OF PHOSPHORUS (P) ENRICHMENT OF SAWGRASS, CLADIUM JAMAICENSE, COMMUNITIES IN THE FLORIDA EVERGLADES

INDICATOR	Response		RESPONSE TIME			
	Unfertilized (no P)	Fertilized (4.8 g P/m²/yr)				
Level I						
Stem height ¹		—	1-2 years			
Leaf P (µg/g)	210	530	<1year			
Leaf C:P (wt:wt)	2100	840	<1 year			
Leaf N:P (wt:wt) ²	29	12	<1 year			
Level II						
Aboveground biomass (g/m²)	1160	2950	1-2 years			
Standing dead biomass (g/m²)	1520	3670	3			
Standing dead P (µg/g)	74	398	3			
Standing dead C:P (wt:wt)	6000	1100	3			
Standing dead N:P (wt:wt) ⁴	51	11	3			
Resorption efficiency (P)	54%	7%	3			
Resorption proficiency (P, %) ⁵	.007	.04	3			
NUE(p)	13500	2500	1-2 years			
Nutrient-intolerant species (g/m²)						
(Utricularia) ⁶	200	30-50	<1 year			
Nutrient-tolerant species (g/m²)						
(Chara) ⁶	0	70-180	1-2 years			
(Acrostichum danaeifolium) ⁷	0	400	1-2 years			
(Typha) ⁷	45	60	No change			
(Lemna) ¹		—	No change			

¹ Determined by visual inspection.

 2 Leaf N content of unfertilized and fertilized plots was 0.60% and n.65%, respectively.

³ Measured during year 3 of the study.

⁴ Standing dead N content of unfertilized and fertilized plots was 0.38% and 0.45%, respectively.

⁵ Same as standing dead P.

6 Slough community.

7 Mixed sawgrass-cattail community.

Notes: The response to P was measured over a 4-year period. In this study, there was no response to P additions. Numerical values in **bold** indicate that phosphorus-fertilized and unfertilized plots were statistically different (p<0.05) from each other.

Source: Data are from Craft et al. 1995, Chiang et al. 2000.

Results from the Everglades experiment indicate that (1) reliable vegetation-based indicators of P enrichment exist and (2) some indicators (e.g., leaf P, Utricularia) respond more rapidly to increased P than others. The rapid and consistent responses of leaf P, NPP, and Utricularia suggest that these indicators are reliable for monitoring conditions in the Everglades. Other indicators, like cattail and duckweed encroachment, were not observed during the first 4 years of the experiment. The absence of cattail encroachment into the fertilized plots probably reflects the fact that competitive displacement is a time-dependent process (Mal et al. 1997). A shift in emergent species composition in the fertilized plots might take longer than the 4-year period of record of the experiment. Furthermore, the reported increase of cattail and duckweed in eutrophic areas of the Everglades may be the result of increased water depth in addition to P enrichment (Urban et al. 1993).

ESTUARINE SALT MARSHES

Like Everglades vegetation, salt marsh vegetation responded quickly when the primary limiting nutrient, in this case N, was added. Leaf N and aboveground biomass of Spartina alterniflora, the dominant species in east coast salt marshes, increased during the first year of N additions (Table 4). Leaf C:N, standing dead N, and $NUE_{(N)}$ also increased relative to unfertilized plots during the first year of N fertilization (Table 4). Stem height, a Level I indicator, also increased in response to N additions, although the response was not significantly different from unfertilized plots. Because nitrogen is a component of chlorophyll, where photosynthesis takes place, N additions quickly translate into increased aboveground biomass. Many studies report that leaf N and aboveground biomass increase within a few months after N is added (Valiela and Teal 1974, Broome et al. 1975, Chalmers 1979). Additions of N sometimes produce more flowering stems of Spartina whereas P additions increase the number of flowering stems even more (Broome et al. 1975).

WET SEDGE TUNDRA

Nutrient additions to wet sedge tundra communities in Alaska revealed that tundra vegetation responded to additions of either N, P, or N+P (Shaver et al. 1998) (Table 5). After 5 years of fertilization, leaf N and aboveground biomass increased in response to N additions, whereas C:N and N:P decreased in N-treated plots. The effect of P additions on functional indicators of enrichment was even more pronounced than with N. Leaf P and aboveground biomass increased dramatically in response to P additions whereas leaf C:P and N:P decreased in P-treated plots (Table 5). The plant response to N+P additions was greater as compared with either N or P applied singly, indicating that tundra communities are limited primarily by P and secondarily by N (Shaver et al. 1998). In a separate fertilization experiment, Shaver and Chapin (1995) added N, P, and K to moist tussock and wet sedge vegetation in the Alaskan tundra. Similar to the Everglades case study, leaf N and P increased following the first year of nutrient additions. Increased plant growth and biomass production was not observed until year 2 of the study and, in year 3, flowering increased in response to fertilizer additions.

In salt marshes and amid tundra vegetation, leaf N concentrations declined after the first year of N fertilization, as N was "diluted" by enhanced biomass production (Valiela and Teal 1974, Shaver and Chapin 1995). However, loss of sensitivity of this Level I indicator was offset by increased NPP, aboveground biomass, and stem height. In contrast to N, in ecosystems where P was limiting or co-limiting, leaf P concentrations remained elevated even as NPP increased in response to P enrichment (Shaver and Chapin 1995, Chiang et al. 2000).

TABLE 4: LEVEL I AND LEVEL II INDICATORS OF NITROGEN (N) ENRICHMENT OF SPARTINA ALTERNIFLORA DOMINATED SALT MARSHES ALONG THE ATLANTIC (NC, GA) COAST

Indicator	Response		Response Time			
	Unfertilized	+N				
Level I	Level I					
Stem height (cm) ¹	77	103	<1 year			
Leaf N (%) ¹	0.82	1.05	<1 year			
Leaf C:N (wt:wt) ^{1,2}	49	38	<1 year			
		•				
Level II	Level II					
Aboveground biomass (g/m ²) ¹	360	600	<1 year			
Standing dead N (%) ^{3,4}	0.90	1.05	<1 year			
NUE _(N)	111	95	<1 year			

 1 From Broome et al (1975), N added as ammonium sulfate at a rate of 16.2 g N/m 2 /yr.

² Leaf C is assumed to be 40%.

³ From Chalmers (1979), N added as sewage sludge at a rate of 2 g N/m ²/wk.

⁴ Same as Resorption Proficiency.

Notes: In this study (Broome et al. 1975), N was the primary limiting nutrient and P (data not shown) was secondarily limiting. Numerical values in bold indicate that phosphorus-fertilized and -unfertilized plots were statistically different (p<0.05) from each other.

The results of the Everglades, salt marsh, and tundra fertilization studies demonstrate that increased leaf nutrient (N, P) content is a powerful indicator of eutrophication because it is among the first to respond to nutrient enrichment. Level I (stem height) and II (aboveground biomass) metrics of NPP likewise respond relatively quickly to nutrient enrichment. They, too, are useful detectors of incipient eutrophication of wetlands.

TABLE 5: LEVEL I AND LEVEL II INDICATORS OF NITROGEN (N) AND PHOSPHORUS (P) ENRICHMENT OF A WET SEDGE TUNDRA (ERIOPHORUM ANGUSTIFOLIUM) IN ALASKA AFTER 5 YEARS OF FERTILIZATION

Indicator		Response				
	Unfertilized (no N or P)	+N (10 g/m²/yr)	+P (5 g/m²/yr)	N+P		
Level I						
Leaf N (%)	1.48 a	1.78 b	1.46 a	1.69 b	<1 yr	
Leaf P (ug/g)	830 a	1120 b	2660 b	3870 b	<1 yr	
		-		-		
Leaf C:N (wt:wt) ²	27.0	22.5	27.4	23.7	<1 yr	
Leaf C:P (wt:wt) ²	482	357	150	103	<1 yr	
Leaf N:P (wt:wt) ^{2,3}	17.8	15.9	5.5	4.4	<1 yr	
		•	•			
Level II						
Aboveground biomass (g/m ²)	142 a	195 b	283 c	404 d	1-2 yr	

¹ Response time was based on an earlier study by Shaver and Chapin (1995).

 2 No statistical analyses were performed on these parameters.

³ Leaf C was assumed to be 40%.

Notes: In this study, indicators of nutrient enrichment responded to additions of either N, P, or N+P, indicating that both N and P limit plant productivity of this community. Numerical values within the same row separated by the same letter were not statistically different (p<0.05) from each other.

Source: Data are from Shaver et al. 1998.

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GLOSSARY

Ecological integrity The capacity of an ecosystem to sustain essential life support services such as energy flow, biogeochemical cycling, niche space, and habitat.

Functional indicators Attributes that describe ecosystem function, like energy flow (e.g., productivity) and biogeochemical (e.g., nitrogen, phosphorus) cycling.

Nutrient resorption efficiency (r) Amount of nutrients (e.g., N or P) resorbed from mature leaves divided by maximum nutrient pool in mature leaves (expressed as g/m^2).

Nutrient resorption proficiency The absolute or lowest levels to which nutrient concentrations are reduced in senesced (dead) leaves.

Nutrient use efficiency (NUE) Aboveground biomass production (e.g., g litterfall/m²) divided by quantity of nutrient (e.g., $g N/m^2$) in litterfall.

Structural indicators Attributes that describe community-level characteristics of ecosystems like species richness, species diversity, and canopy architecture (e.g., stem height, vertical stratification).