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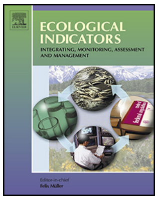

Vegetation and microbial indicators of nutrient status: Testing their consistency and sufficiency in restored calcareous wetlands

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ABSTRACT

Various indicators have been used to diagnose nutrient status of an ecosystem, but their consistency among different ecosystem components (e.g., soil microbes and plants) remains rarely examined. In this study, we selected three sites with a gradient of phosphorus (P) concentrations, i.e., two P-enriched restored sites and an oligotrophic, P-limited reference site in the Hole-in-the-Donut of Florida Everglades, USA. Three sets of indicators for plants, soils microbes, and periphyton were measured in February, 2010. For vegetation indicators, TN:TP ratio indicates P-limitation but N use and resorption efficiency suggest N limitation at the two restored sites. For soil microbial indicators, N- and P-acquiring enzyme activities (i.e., leucine aminopeptidase and alkaline phosphatase) indicate N and P limitation at the restored sites and the reference sites, respectively. For periphyton indicators, significantly higher nitrogenase activities suggest N limitation at the restored sites. Overall, soil microbial and periphyton indicators consistently showed N limitation at the two restored sites. Furthermore, the microbial enzyme activities were significantly correlated with soil/periphyton chemical properties (e.g., soil extractable inorganic and organic N and P, and TN:TP ratio of soil and periphyton) and were more powerful in diagnosing the nutrient status. The inconsistency between vegetation-based indicators and microbial indicators not only suggests the insufficiency of a single indicator, but also indicates the heterogeneity in nutrient limitation of different components of an ecosystem. It is important to evaluate those indicators in order to give implications for restoration, in particular in those ecosystems that are sensitive to even small changes of nutrient availability.

1. Introduction

Nutrient status for an ecosystem is generally described as eutrophic (enriched) or unenriched by specific nutrients (U.S. EPA, 2002). For most ecosystems when enriched by one element, they are more likely to be limited by another element. Many ecosystems on earth are characterized by low nutrients and high species diversity. Two typical examples are heathlands with acidic soils and calcareous ecosystems with alkaline soils (Gibson and Brown, 1991; Kiehl and Pfadenhauer, 2007; Mitchell et al., 2000; Niinemets and Kull, 2005; Diaz et al., 2008; Piqueray et al., 2011). Plants in such nutrient-poor ecosystems can be very sensitive even to small changes in nutrient availability (Fischer and Stöcklin, 1997). Agriculture is a major human disturbance that inputs excess nitrogen (N) and phosphorus (P) to these fragile ecosystems, causing nutrient problems and invasion of exotic species (Bending et al., 2000; Davidson et al., 2007; Hausman et al., 2007; Kalinina et al., 2009; Hamilton and Landman, 2011).

A diversity of indicators have been used to describe nutrient status of different components (e.g., water, soil, and plants) in an ecosystem (Hill et al., 2006; Craft et al., 2007; Corstanje et al., 2007, 2009; Davidson et al., 2007; Inglett and Inglett, 2013). For example, vegetation responds to nutrient variations by changing biomass production, species composition, and nutrient uptake (Craft et al., 2007). Foliar TN:TP ratios, plant biomass, and nutrient use/resorption efficiency have been used as indicators of nutrient limitation to primary production (van den Driessche, 1974; Vitousek, 1982; Koerselman and Meuleman, 1996; Aerts et al., 1999; Niinemets and Kull, 2005; Craine and Jackson, 2010). Microbial indicators (e.g., microbial biomass, respiration, and extracellular enzyme activities) can rapidly respond to changing nutrient status and have been increasingly utilized as sensitive indicators of ecosystem restoration and nutrient enrichment (Olander and

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Vitousek, 2000; Corstanje and Reddy, 2006; Harris, 2003, 2009; Hill et al., 2006; Sinsabaugh et al., 1993, 2009).

Despite recognition of various indicators of nutrient status, the consistency of different indicators in describing nutrient status of an ecosystem has not been previously examined (Craft et al., 2007; Corstanje et al., 2009; Piqueray et al., 2011; van Katwijk et al., 2011). The sensitivity to nutrient changes may vary between different components (e.g., soil, microbes, periphyton, and plants). For example, soil chemical characteristics and vegetation tend to change gradually as a result of the input of nutrients, while soil microbes may exhibit distinct, abrupt changes (Corstanje et al., 2007; Craft et al., 2007). Moreover, the theory of differential nutrient limitation has been recently discussed that different species groups in a given ecosystem are not necessarily limited by the same nutrients (Sundareshwar et al., 2003; Lang et al., 2012; Hartman and Richardson, 2013). More evidence is needed to test its generality.

The Florida Everglades is extremely oligotrophic and sensitive to small increases in P concentrations (Noe et al., 2001). Over the last century, the system has been severely impacted by human activities, resulting in P enrichment and invasion of exotic species (Sklar et al., 2005). In the areas with high P loading it has been observed that a shift from P to N limitation occurs (Inglett et al., 2011). Significant efforts have been made to restore the ecosystem and reduce agricultural and urban nutrient loading. Accordingly, it is vital to identify and develop a suite of system-wide indicators to monitor the nutrient status and evaluate the restoration. Vegetation, periphyton (i.e., a consortium of algae, cyanobacteria, heterotrophic microbes, and detritus that are attached to submerged surfaces), soil biogeochemical properties, and microbial variables, have been used to monitor nutrient status in the Everglades (Miao and Sklar, 1997; U.S. EPA, 2002; Qian et al., 2004; Corstanje et al., 2009; Gaiser, 2009; Inglett et al., 2009). For example, periphyton-based metrics and microbial indicators were considered as early warning signals for P enrichment (Qian et al., 2004; Gaiser, 2009; Corstanje et al., 2009). However, few studies have presented the nutrient status of multiple components at one time in the same region of Everglades.

In this study, we selected three calcareous wetlands with a gradient of P concentration in Southern Everglades. Three sets of indicators for soil microbes, vegetation, and periphyton were measured. Our objectives were to investigate (1) how different components respond to the changing nutrient status, (2) whether the indicators of vegetation, soil microbes, and periphyton consistently tell the same nutrient status, (3) whether different components are limited by different nutrients.

2. Materials and methods

2.1. Study site

The Hole-in-the-Donut (HID) located in the Everglades National Park, Florida, USA, is a limestone-based calcareous wetland with low nutrients. Historic farming enriched soil P; and after farming ceased in 1975, Brazilian pepper (*Schinus terebinthifolius*) invaded and dominated (Li and Norland, 2001). To restore HID to natural sawgrass (*Cladium jamaicense* Crantz) ecosystems, various methods were tried (Doren et al., 1991) with complete soil removal down to the bedrock being the most efficient to remove *Schinus* (Dalrymple et al., 2003). Through complete soil removal, restored sites tend to experience a primary succession. At the first stage, periphyton are the primary components of the ecosystem, facilitating soil formation and fixing atmosphere N_2 (Inglett and Inglett, 2013). Native plants then gradually reestablish at the restored sites. During the restoration, different restored sites with different “restored age” formed a gradient of P concentrations. Inglett and Inglett (2013) investigated the biogeochemical changes during 16 years after complete soil removal in a formerly farmed wetland in this area, and they found that restored sites shifted from initial N limitation toward a state of co-limitation by N and P, and then mimic the natural ecosystem with P limitation.

Two sites that were restored in the year of 2000 and 2003 (termed as Res00 and Res03, respectively), as well as an unfarmed reference site (termed as Reference) adjacent to the restored areas, were selected in the HID region of Everglades National Park (Fig. 1). At each site, we identified five sampling stations (Fig. 1). Soil depth varied among the three sites, with deeper marl soils (Biscayne and Perrine series) at the reference site (10 cm) and shallower soils (2–3 cm) at the restored sites that have been developing since soil removal (Smith et al., 2011).

2.2. Sampling methods

In February 2010, three composite samples of surface soil and periphyton were collected at each of the 15 sampling stations. Live healthy leaves of the dominant vegetation were collected by hand at the 15 locations. Not all species were sampled, but an effort was made to include species that were present at all sites. The targeted species included representatives of the genera *Muhlenbergia*, *Cladium*, *Typha*, *Andropogon*, and *Schinus* (Dalrymple et al., 2003). All samples were sealed in plastic bags and kept on ice until their return to the laboratory where the samples were refrigerated at 4 °C until subsequent analysis. Soil samples were sieved to remove roots and rock fragments greater than 2 mm diameter. Sieved soil samples were used to determine all microbial and enzyme related parameters, while a subsample of sieved soil was oven dried at 105 °C for 3 days and ground using a mortar and pestle to quantify moisture content and total nutrients. Plant tissues and periphyton were oven dried at 65 °C for 3 days and ball milled to quantify total nutrients.

In April, 2010, we clipped aboveground plant biomass in four 1 m × 1 m plots at two locations at each of the three sites (Fig. 1). Dead and live parts were separated, and oven dried at 65 °C for 3 days and ball milled to quantify total nutrients.

2.3. Plant biomass and nutrient-use/resorption efficiency

The NUE was generally termed as the amount of organic matter produced per unit of nutrient taken up (Vitousek, 1982). The NRE is defined as the ratio of the amount of nutrients resorbed from senescing leaves to the maximum nutrient pool in the senescing leaves (Aerts et al., 1999). The NUE and NRE of N and P were determined following methods outlined by Berendse and Aerts (1987), Aerts et al. (1999), and Feller et al. (2002). The NUE is calculated as:

$$\mathrm{NUE}\,(\mathrm{g\ biomass\ mg^{-1}\,N}) = A/L_n,$$

where A is N productivity, the dry matter production per unit of N, and L_n is the N requirement per unit of N in the plant. A and L_n are calculated as:

$$A\,(\mathrm{g\ dry\ wt\ mg^{-1}\,N}) = \mathrm{biomass\ production}\,(\mathrm{g\ dry\ wt\ m^{-2}yr^{-1}})/\ \mathrm{biomass\ N}\,(\mathrm{mg\ N\ m^{-2}yr^{-1}}),$$

$$L_n\,(\mathrm{unitless}) = N_{live}\,(\mathrm{mg\ m^{-2}})/N_{senescent}\,(\mathrm{mg\ m^{-2}}),$$

where N_{live} and $N_{senescent}$ are the amounts of N in the live and senescent fractions of biomass.

The NUE at the ecosystem level was taken as biomass production per unit of N in senescent leaves (g dry wt biomass $\mathrm{mg^{-1}}$ N).