

## Research Letter

# Assessment of Nutrient Limitation in Floodplain Forests with Two Different Techniques

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We assessed nitrogen and phosphorus limitation in a floodplain forest in southern Georgia in USA using two commonly used methods: nitrogen to phosphorus (N:P) ratios in litterfall and fertilized ingrowth cores. We measured nitrogen (N) and phosphorus (P) concentrations in litterfall to determine N:P mass ratios. We also installed ingrowth cores within each site containing native soil amended with nitrogen (N), phosphorus (P), or nitrogen and phosphorus (N + P) fertilizers or without added fertilizer (C). Litter N:P ratios ranged from 16 to 22, suggesting P limitation. However, fertilized ingrowth cores indicated N limitation because fine-root length density was greater in cores fertilized with N or N + P than in those fertilized with P or without added fertilizer. We feel that these two methods of assessing nutrient limitation should be corroborated with fertilization trials prior to use on a wider basis.

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## 1. INTRODUCTION

Many ecologists have applied Liebig's law of the minimum to forest ecosystems and have sought to determine what nutrient limits net primary production (NPP). Globally, NPP generally is considered to be limited by nitrogen (N) [1], but many forests may be switching from nitrogen to phosphorus limitation because humans have increased atmospheric nitrogen inputs to forests through fossil fuel combustion, nitrogen fertilizer production, and other human activities [2]. Assessing nutrient limitation, however, has been historically problematic because it typically has required laborious and time-consuming fertilization trials [3]. Within the last decade, ecologists have increasingly used simpler methods to determine nutrient limitation in forests, such as foliar N:P ratios [4–11] or root growth into fertilized ingrowth cores [12–18], but no study to date has explicitly compared these two techniques.

The root ingrowth core method was originally developed to measure root production and involves excavating a soil core that is replaced with root-free growth medium (e.g., native soil, vermiculite, peat), sometimes enclosed in a mesh

bag [18]. The core is extracted after a given period of time and roots growing into the core are removed. The ingrowth core technique was modified by Cuevas and Medina [13] to measure nutrient limitation by comparing root growth into cores fertilized with different nutrients. Their modification makes use of the well-known plastic response of plants to proliferate roots into nutrient-rich patches [19] and assumes that roots respond more strongly to limiting nutrients. Raich et al. [17] corroborated this technique in forests with known nutrient limitation, and it has recently come into widespread use as a result of their work [12, 14–16, 18].

N:P ratios in plant tissue have been used as diagnostic indicators of N or P limitation, and thresholds have been established for some ecosystems [3, 9, 20]. The use of N:P ratios to determine N or P limitation is based on the assumptions that either N or P is limiting and that N or P concentration in plant tissue reflects nutrient deficiencies in soils. For wetland plant communities in Europe, Koerselman and Meuleman [3] suggested that communities with an N:P ratios >16 were P-limited while those with an N:P ratios <14 were N-limited. Lockaby and Conner [20] extended this work to forested wetlands of the southeastern United States

by examining N:P ratios in litterfall and proposed that N:P ratios <12 were N-limited and those >15 were P-limited.

Here, we used the fertilized ingrowth core and litter N:P ratio techniques to assess nutrient limitation in a floodplain forest along a blackwater stream in southwestern, Ga, USA. Blackwater streams typically have lower inorganic materials and suspended sediments in their waters and therefore floodplain forests associated with these systems generally are thought to be nutrient poor [21]. In other blackwater systems in the southeastern United States, P is generally considered to be limiting [8, 22]. Thus, we expected both techniques would indicate P limitation.

## 2. METHODS

Three 26 × 26 m plots were established within the floodplain forest of the Chickasawhatchee Wildlife Management area of Baker and Calhoun counties, Ga, USA. The overstory was dominated at all sites by *Liquidambar styraciflua* L, *Acer rubrum* L, and various *Quercus* species (*Quercus lyrata* Walt., *Quercus laurifolia* Michx., *Quercus nigra* L). The understory consisted primarily of *Smilax* spp., *Toxicodendron radicans* (L.) Kuntze and *Sabal minor* (Jacq.) Pers. Soils were Typic Alabaqualls (Megget series) or Typic Fluvaquents (Muckalee series) [23, 24]. Mean annual temperature during the study year was 19°C and annual precipitation was 141 cm, which was 11% above normal (National Climatic Data Center, Asheville, North Carolina).

Forty cores of mineral soil (2-cm diameter × 20-cm deep) were collected with a soil probe from each plot. Soil was sieved through a 2-mm mesh screen to remove coarse fragments, combined by plot, and air dried. Soil pH was measured using an Accumet pH meter, and total C and N was determined using a Perkin Elmer Series II CHNS/O analyzer (Perkin Elmer Inc., Boston, Mass, USA). Soils were analyzed for extractable phosphate on a Lachat Quickchem AE autoanalyzer following double-acid extraction [25].

Five 0.25-m<sup>2</sup> litter traps were placed randomly in each plot in September 2003, and litterfall was collected in November 2003 to determine litter N and P concentrations. Litter was dried to a constant mass (60°C), weighed, ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), and pulverized with a ball Spex 8000D ball grinder (SPEX CertiPrep Group, Metuchen, NJ, USA). Litter N concentration was determined using the dry combustion method on a Perkin Elmer Series II CHNS/O analyzer. Litter P was measured with the dry ash method [26] followed by analysis on a Lachat Quickchem AE autoanalyzer (Lachat Instruments, Milwaukee, Wis, USA). Litter N:P ratios were calculated as mass ratios typically used for forest and wetland vegetation.

For the ingrowth core study, each plot was divided into 2 × 2 m grid cells consisting of 196 grid intersections and 44 of the intersections were designated randomly for installation of ingrowth cores. In early July 2003, a 10.16-cm diameter × 20-cm deep core was extracted at each selected location. The core was filled with soil collected from a nearby site that was sieved through a 2-mm screen to remove roots. Soil was packed to approximately the same bulk density as the soil

that was removed. The core was either left unfertilized or had phosphorus, nitrogen, or phosphorus and nitrogen fertilizers mixed into the top 5 cm. Treatments were replicated 11 times per plot. Nitrogen was added as 0.75 g of POLYON coated urea (43-0-0 NPK, Harrell's Inc., Lakeland, Fla, USA), and phosphorus was added as 0.40 g triple superphosphate (0-46-0 NPK, Southern States Cooperative, Christiansburg, Va, USA) to raise soil nutrient availability by 400 kg N/ha and 100 kg P/ha, respectively. Cores were harvested after 4 months in late November 2003 by extracting a 7.62-cm diameter × 20-cm deep core from the middle of each ingrowth core. Seven cores were not collected due to damage by animals. Cores were washed over a 1-mm mesh screen to remove adhering soil particles. Roots were separated from soil organic matter in the lab and subsampled for root length analyses. Each subsample was scanned and analyzed using WinRHIZO software (Regent Instruments, QC, Canada) to determine specific root length (SRL, cm of root per g of root). Root samples were dried to a constant mass at 60°C and weighed. Root length density (km root per m<sup>3</sup> of soil) was calculated by multiplying SRL by root mass. We ran a one-way ANOVA to compare root length density among fertilization treatments with plots as blocking factors using the GLM procedure of SAS version 8 (Statistical Analysis System, Cary, NC, USA). We also used to 95% confidence limits to determine whether N:P were different from threshold ratios for 12 and 15, for N and P limitation, respectively.

## 3. RESULTS AND DISCUSSION

The fertilized ingrowth core method indicated N limitation because root length density in cores fertilized with N or N + P was nearly double that of root length density in P-fertilized cores or in cores with no added fertilizer ( $P < .01$ , Figure 1). In addition, root length density did not differ between P-fertilized cores and cores without fertilizer ( $P < .99$ ). According to 95% confidence intervals, litter N:P ratios (mean = 19.1) were greater than 12 but not than 15, the thresholds for N and P limitation, respectively, in floodplain forests of the southeastern United States [20]. This suggested P limitation or colimitation by N and P in this forest. Low N (0.8%) and P (0.04%) compared to other floodplain forests [20] may support colimitation (Table 1). However, high nutrient resorption proficiency of P, defined by Killingbeck [27] as the degree to which plants can reduce the level of a given nutrient in senescing leaves, lends support for P limitation in this forest. Proficiency was <0.05% for P and >0.7% for N, which suggested nearly complete resorption of P and intermediate resorption of N [27]. The discrepancy between the two techniques is somewhat puzzling. In the following paragraphs, we provide possible reasons for these contradictory results.

We feel there are three likely explanations why roots responded only to N fertilization in potentially P-limited conditions. First, the use of fertilized ingrowth cores to measure nutrient limitation is centered on the assumption that roots respond more strongly to limiting nutrients than to those that are "non" limiting. However, this assumption

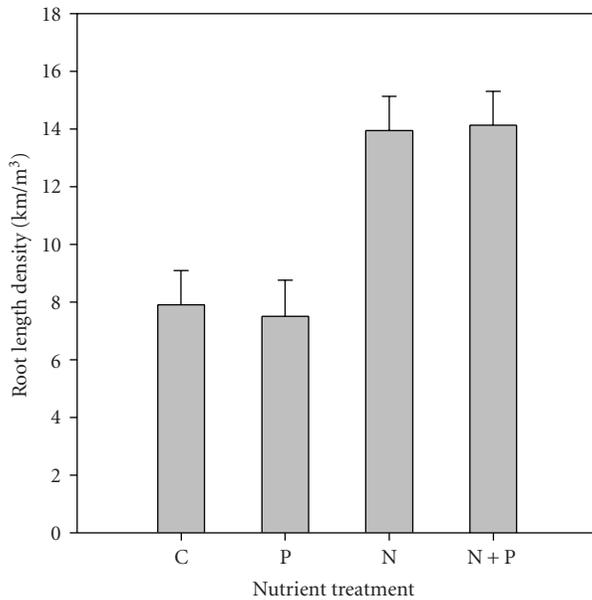


FIGURE 1: Fine root response to fertilized ingrowth cores (+1 SE). C = control, P = phosphorus fertilization with triplesuperphosphate (100 kg P/ha), N = nitrogen fertilization with coated urea (400 kg N/ha), N + P = nitrogen and phosphorus fertilization with triplesuperphosphate, and coated urea (100 kg P/ha + 400 kg N/ha). Different letters indicate significant differences among treatments ( $P < .05$ ).

TABLE 1: Soil and litter chemistry attributes for the three floodplain forest plots in the Chickasawatchee Wildlife Management Area. Values are means with ranges in parentheses.

Attribute	Amount
Soils	
Extractable $\text{PO}_4\text{-P}$ ( $\mu\text{g/g}$ )	1.0 (0.7–1.3)
Total N (%)	0.07 (0.06–0.08)
Total C (%)	1.6 (1.3–1.9)
pH	5.0 (4.2–5.4)
Litter	
P concentration (%)	0.04 (0.03–0.05)
N concentration (%)	0.80 (0.67–0.98)
C concentration (%)	49.8 (49.2–50.8)
N:P ratios	19.1 (16.1–21.7)
C:N ratios	63.7 (50.0–76.0)

may not always be valid for all forests. Hodge et al. [28] suggested that proliferation may be an adaptive response by plants to obtain nutrients when in competition with other plants rather than a response based on immediate nutritional needs. If this is the case, plants may not respond more strongly to limiting nutrients because they are constrained by their evolutionary history. For example, plants species adapted to soils that are historically N-deficient only may have the capacity to proliferate roots into N-rich patches even when P is limiting. Second, the ingrowth core method operates on the implicit assumption that all plants within a

community can proliferate roots into nutrient-rich patches. However, not all plants can proliferate roots [29], and nutritional status of individual species may differ from that of the whole plant community [30]. Therefore, proliferation may be a nutritional response by individual species that may or may not reflect nutrient limitation of the whole community. Third, P may have become rapidly unavailable to plants due to adsorption into Fe and Al complexes or leaching from the cores. The type of fertilizer used in our study, triplesuperphosphate (TSP), is water soluble and fast releasing even though the level of P we used was relatively high (100 kg/ha). Although this may help to explain the lack of response to P, it does not offer any insight as to why roots responded strongly to N fertilization.

There are also several reasons why litter N:P ratios may be poor indicators of P limitation in N-limited conditions. First, the use of N:P ratios to indicate nutrient limitation requires that either N or P limits NPP. N:P ratios cannot detect limitation by another resource (i.e., other nutrient, light, water) or colimitation by multiple resources. The fertilized ingrowth core as used in our study also could not determine nutrient limitation by other resources besides N and P but has the flexibility to at least test limitation or colimitation by other nutrients. Second, assessing nutrient limitation through N:P ratios also necessitates that these ratios are indicators of nutrient deficiencies in soils. However, N:P ratios of individual species do not always vary across a gradient of known nutrient limitation [31], but Aerts and Chapin [30] warned that N:P ratios of individual species may differ from community N:P ratios. Third, thresholds for N or P limitation often are hard to establish. Although there is a general consensus that low N:P ratios indicate N limitation, there is no agreement as to whether intermediate to high N:P ratios indicate P limitation [32]. Some researchers have found N limitation or colimitation by N and P when N:P ratios are high [9, 33]. Finally, all of the above problems may have been compounded by the use of litter N:P ratios instead of N:P ratios in live foliage because of differences in retranslocation efficiencies between N and P. The use of foliar N:P ratios in conjunction with litter N:P ratios may have provided more insight into what nutrient was limiting.

In conclusion, we found that these two techniques gave contradictory results as to what nutrient was limiting (i.e., N or P) and therefore cannot be used to reliably indicate nutrient limitation for all forested ecosystems. We feel both techniques should be corroborated with fertilization trials prior to use on a wider basis.

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