

Phosphorus Limitation of Coastal Ecosystem Processes

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Primary production in coastal wetlands is conventionally thought to be limited by nitrogen. Although the plant community in a pristine salt marsh was found to be limited primarily by nitrogen availability, the bacterial community in the soil was limited by phosphorus. Hence, in coastal wetlands, and possibly in many ecosystems, individual trophic groups may respond differently to nitrogen and phosphorus loading. Phosphorus limitation of the growth of nitrogen-transforming bacteria will affect carbon fixation, storage, and release mediated by plants, a result that has important implications for ecosystem management.

It has been well established that nitrogen is a major nutrient that limits primary production in the coastal zone (1), including in salt marsh ecosystems. However, responses of other ecosystem processes, such as microbial respiration or microbial transformation of nitrogen, have not been as well studied in relation to nitrogen availability. Here, we provide evidence for differential nutrient limitation of autotrophs and microbes in a pristine coastal wetland. These results complicate our ability to predict ecosystem responses to nutrient loadings.

In recent decades, human activities have increased the availability of nutrients such as nitrogen (1) and phosphorus (2). Changes in the nutrient loadings to ecosystems affect carbon and nutrient transformations. For example, experimental nutrient additions are known to affect nitrogen fixation (3) and denitrification (4), the growth efficiency of heterotrophic bacteria (5), and a multitude of ecosystem processes. Predicting the responses of ecosystems to nutrient loading is a challenge, because there are multiple factors that regulate biogeochemical transformations (4, 6). The differential responses to nutrients found among trophic groups and their subsequent interactions limit our predictive capability. For example, nitrogen inputs may accelerate carbon fixation by primary producers, whereas other nutrients like phosphorus may affect carbon turnover by heterotrophs (7).

The extent to which nutrient enrichment in an ecosystem alters biogeochemical processes

depends on the relative nutrient status of autotrophs and decomposers and on the elemental ratios in available organic substrates. To investigate how the relative nutrient status of two important trophic groups regulates the dynamics of nitrogen and phosphorus within an ecosystem, we used above-ground biomass (8), bacterial numbers (9), bacterial thymidine incorporation (10), and pore-water phosphatase activity (11) as indicators of macrophyte and bacterial response to fertilization (12) in a pristine coastal salt marsh in South Carolina.

Our results are consistent with studies (13, 14) showing that plant primary production in marine systems is limited primarily by nitrogen availability. Macrophyte production responded positively to nitrogen amendments, but not to singular additions of phosphorus, and showed the greatest response when nitrogen and phosphorus were added together (Fig. 1A) (table S1). This result indicates that the macrophytes were limited primarily by nitrogen availability and secondarily by phosphorus availability. However, the pore-water phosphatase activity was lowest in the phosphorus-only treatment, which indicates that some component of the ecosystem was phosphorus limited (Fig. 1B). A direct count of sediment bacteria in the rooting zone showed that the bacterial numbers were higher in phosphorus-treated plots (Fig. 1C). Although plant primary production was stimulated by nitrogen and then phosphorus, bacterial processes did not appear to be stimulated by nitrogen alone. It appeared that, in contrast to the macrophytes, the bacterial community was limited by phosphorus and not by nitrogen.

Phosphorus limitation of bacterial activity was confirmed in a controlled experiment in which sediments from unfertilized marsh plots were amended with either nitrogen or phosphorus in the laboratory. Bacterial production, estimated by thymidine incorporation, was signif-

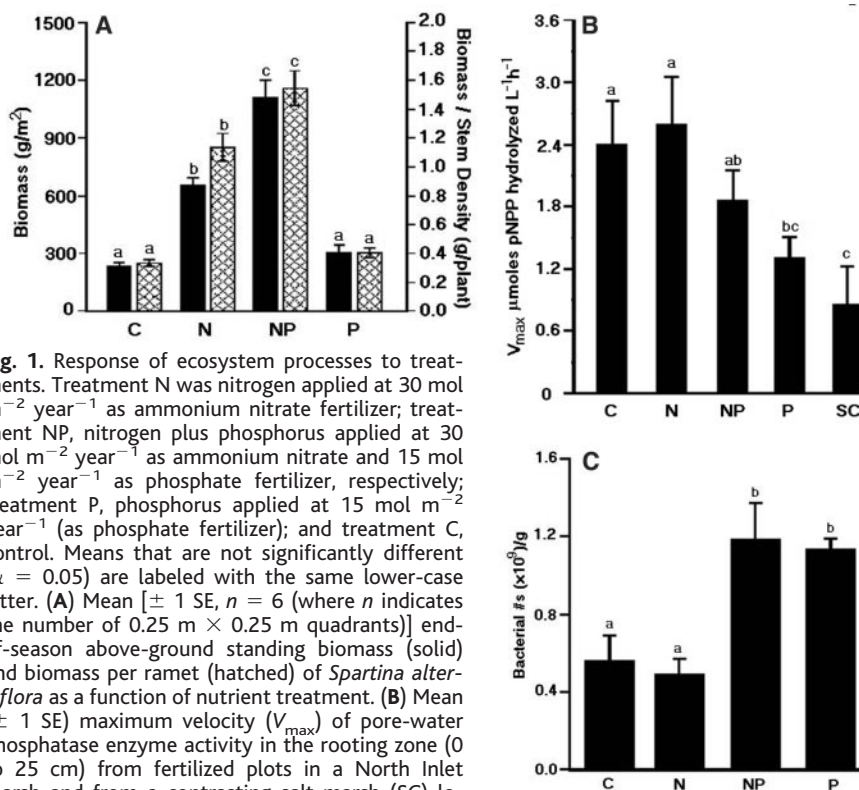


Fig. 1. Response of ecosystem processes to treatments. Treatment N was nitrogen applied at 30 mol m⁻² year⁻¹ as ammonium nitrate fertilizer; treatment NP, nitrogen plus phosphorus applied at 30 mol m⁻² year⁻¹ as ammonium nitrate and 15 mol m⁻² year⁻¹ as phosphate fertilizer, respectively; treatment P, phosphorus applied at 15 mol m⁻² year⁻¹ (as phosphate fertilizer); and treatment C, control. Means that are not significantly different ($\alpha = 0.05$) are labeled with the same lower-case letter. (A) Mean [± 1 SE, $n = 6$ (where n indicates the number of 0.25 m \times 0.25 m quadrants)] end-of-season above-ground standing biomass (solid) and biomass per ramet (hatched) of *Spartina alterniflora* as a function of nutrient treatment. (B) Mean (± 1 SE) maximum velocity (V_{max}) of pore-water phosphatase enzyme activity in the rooting zone (0 to 25 cm) from fertilized plots in a North Inlet marsh and from a contrasting salt marsh (SC) located at the mouth of the urbanized Cooper River estuary in South Carolina. Enzyme activity was assayed spectrophotometrically (with the use of p-nitrophenylphosphate as substrate) (11) in triplicate at in situ pH monthly for 10 months. (C) Effect of nutrient treatments on mean (± 1 SE, $n = 3$) bacterial abundance in surface sediment (0 to 5 cm).

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icantly ($P < 0.05$) higher in the phosphorus-enriched treatment than in nitrogen-enriched treatments (Fig. 2A), demonstrating that soil bacteria in this wetland were limited by phosphorus availability. Although carbon amendments to sediments failed to stimulate bacterial production [Supporting Online Material (SOM) Text], carbon amendments to sediments from phosphorus-treated plots further stimulated bacterial production (Fig. 2B). The bacterial community in this pristine marsh was limited primarily by the availability of phosphorus and secondarily by the availability of labile carbon.

We found that phosphorus limitation of microbial heterotrophs has the potential to increase the loss of nitrogen and to alter ecosystem-level inputs and outputs of nitrogen. For instance, the potential rate of denitrification as measured by the acetylene block assay (12) was higher when phosphorus availability was limited (Fig. 3A). The potential rate of N_2O flux decreased from $3.3 \text{ nmol g}^{-1} \text{ h}^{-1} \pm 0.07$ (SD) when phosphorus was limiting to $0.7 \text{ nmol g}^{-1} \text{ h}^{-1} \pm 0.2$ when samples were enriched with $200 \mu\text{M}$ phosphorus (Fig. 3A). Thus, the higher

rate of N_2O production when phosphorus was limiting is indicative of increased loss of nitrogen via the denitrification pathway. Although nitrification may contribute more N_2O , a gaseous intermediate in both nitrification and denitrification pathways (15), than denitrification under aerobic conditions (16), our experiments were carried out under anoxic conditions and at saturating concentrations of nitrate, which should prevent nitrification and its contribution to the measured N_2O production. The greater flux of N_2O under phosphorus-limiting conditions was a function of higher N_2O production and was not because of differences in the rates of conversion of N_2O to N_2 . N_2O production, measured without the addition of acetylene, was also higher under phosphorus-limiting conditions (Fig. 3B). Thus, phosphorus limitation of microbial heterotrophs in coastal environments may result in the loss of excess nitrogen through denitrification.

Nutrient additions to marsh sites altered the rates of heterotrophic nitrogen fixation. For example, rates of potential nitrogen fixation (12) were completely inhibited in plots fertilized with nitrogen (Fig. 3C). Phosphorus additions to marsh sites at North Inlet also altered the

rates of heterotrophic nitrogen fixation, thus having an indirect effect on primary production. Although phosphorus enrichment of temperate coastal waters enhances nitrogen fixation in the water column (17), phosphorus enrichment of our study site did not stimulate nitrogen fixation. Whereas phosphorus enrichment has been shown to stimulate nitrogen fixation by legumes (18) and cyanobacteria (19), heterotrophic nitrogen fixation in phosphorus-amended plots was inhibited in this coastal wetland (Fig. 3C) (SOM Text).

Our data illustrate the complex interactions among phosphorus, nitrogen, and carbon cycles. Like the field study, marsh sediments taken from control sites that were amended with phosphorus in the laboratory also showed a reduction in heterotrophic nitrogen fixation (Fig. 3, C and D). The phosphorus-mediated reduction in heterotrophic nitrogen fixation observed here is most likely a function of secondary carbon limitation (Fig. 2B). Because nitrogen fixation is energetically expensive and is often limited by the availability of suitable carbon substrates (20), glucose additions to marsh sediments enhanced the endogenous rates of heterotrophic nitrogen fixation (21). In our study too, singular

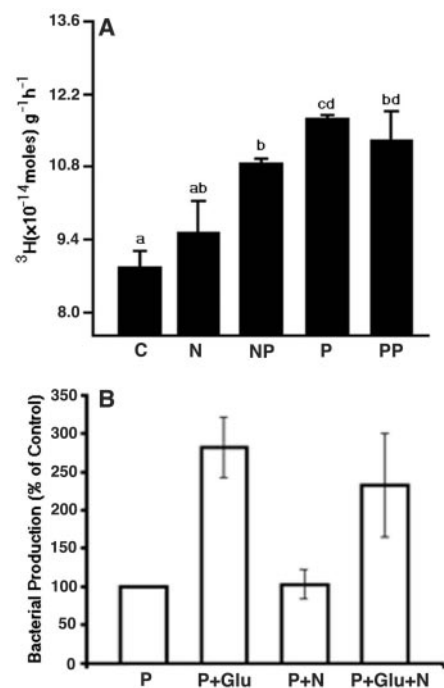


Fig. 2. (A) Effects of nutrient treatments on rates (mean ± 1 SE, $n = 6$) of thymidine incorporation into bacteria in North Inlet salt marsh sediment. Treatments N, NP, and P were nitrogen, nitrogen plus phosphorus, and phosphorus, respectively, whereas treatment PP was a phosphorus amendment in the form of pyrophosphate. (B) Rates (mean ± 1 SE, $n = 6$) of thymidine incorporation into bacteria in sediments fertilized in the field with phosphorus. Treatment P was a control (unaltered in the lab); treatment P+Glu, an addition of glucose in the lab; treatment P+N, an addition of nitrogen; and treatment P+Glu+N, an addition of glucose and nitrogen.

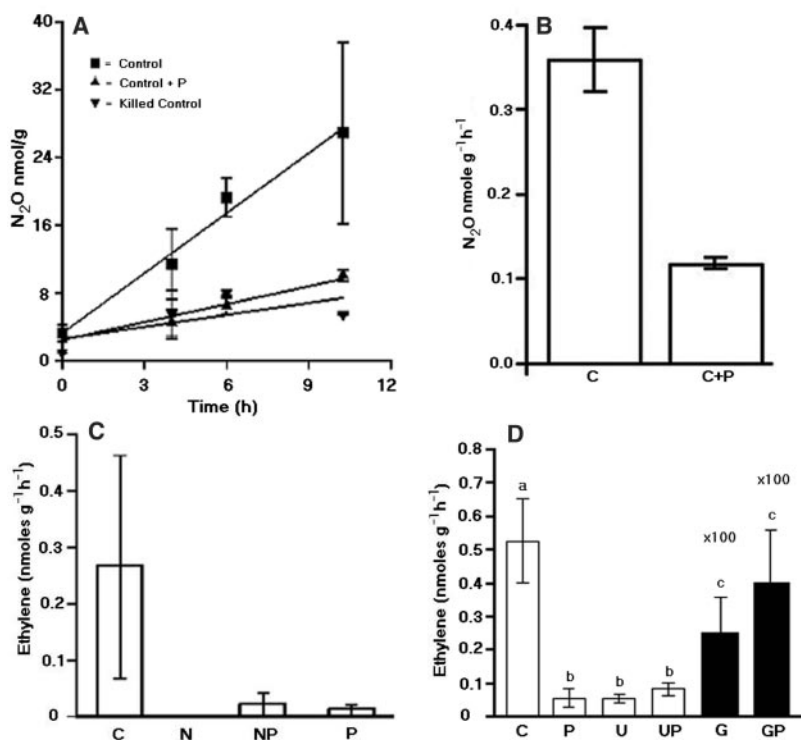


Fig. 3. (A) Rates (mean ± 1 SD, $n = 2$) of potential denitrification (acetylene block) per weight of dry sediment in laboratory incubations of sediment from field controls amended with nitrate (5 mM final concentration) either with or without added phosphorus ($200 \mu\text{M}$). (B) Rates (mean ± 1 SD, $n = 2$) of potential N_2O production (without acetylene) per weight of dry sediment in laboratory incubations of sediment amended with nitrate (20 mM final concentration) either with or without added phosphorus ($400 \mu\text{M}$). (C) Rates (mean ± 1 SE, $n = 6$) of nitrogen fixation in field controls (C) and in field treatments as in Fig. 1. (D) Rates (mean ± 1 SE, $n = 3$) of nitrogen fixation in sediments from field controls amended in the lab with glucose (G), urea (U), or phosphorus (P). All additions were made to 10 mM final concentration. Nitrogen fixation rates were greater in the presence of glucose (solid bars) than without. Multiply the scale by 100 for bars labeled " $\times 100$." Significance of treatment effects, determined on log-transformed data, is denoted by different letters.

additions of glucose to homogenized rhizosphere sediments increased heterotrophic nitrogen fixation (Fig. 3D). However, the response was amplified when glucose plus phosphorus were provided, in contrast to the response when phosphorus alone was added (Fig. 3D). This result implies that phosphorus loading to coastal environments can induce carbon limitation for microbial processes.

Simultaneous additions of urea and phosphorus failed to stimulate nitrogen fixation (Fig. 3D), unlike the response to phosphorus plus glucose, probably because hydrolysis of urea yields carbon in a form that does not support microbial growth. However, heterotrophic nitrogen fixation by root-associated diazotrophs was also inhibited when other forms of dissolved organic nitrogen (e.g., amino acids) were provided as a readily bioavailable carbon source (22). There is evidence that bacteria can use amino acids as sources of both carbon and nitrogen (23). Hence, microbial processes such as nitrogen fixation are influenced by the quality of bioavailable carbon, with important implications for the regulation of primary productivity.

Our results in coastal wetlands demonstrate that phosphorus limitation of microbial growth will impact the transformation and availability of nitrogen, which can influence carbon fixation, storage, and release. Whereas nitrogen amendments increased the primary production of marsh macrophytes, simultaneous additions of nitrogen and phosphorus at our site increased soil respiration and carbon turnover (24), because microbial heterotrophs here are limited primarily by phosphorus and secondarily by available carbon. This has important management implications. For example, whereas hypoxic events in temperate coastal waters are often attributed to eutrophication due to nitrogen loading (1), phosphorus enrichment of samples taken from black-water rivers has been shown to increase biological oxygen demand (7), which is consistent with an interpretation of phosphorus limitation of microbial heterotrophs. Hence, it is not prudent to manage ecosystems solely on the nutrient response of primary producers or to ascribe the regulation of ecosystem processes to the availability of a single limiting nutrient.

Although differential nutrient limitation among trophic groups has not been explicitly studied elsewhere, there is evidence from other marine (25, 26), estuarine (27), and tropical (28) and temperate (29) forest ecosystems for phosphorus limitation of heterotrophs and for differential nutrient limitations of autotrophs and microbial heterotrophs in rivers (7). Thus, differential nutrient limitations observed in marshes are also likely to occur in other ecosystems, possibly as a consequence of trophic-level variation in the biomass C:P ratio that varies inversely with specific growth rate (30). Moreover, differential nutrient limitation should affect the balance of energy and elements in living

systems, or biological stoichiometry (30), and the development of the ecosystem through competition for nutrient resources (31). Differential nutrient limitation of primary producers and microbial heterotrophs likely represents a consequence of ecosystem development that maximizes overall resource utilization and conservation.

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Supporting Online Material

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Materials and Methods
SOM Text
Fig. S1
Table S1

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The Cellular and Molecular Origins of Beak Morphology

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Cellular and molecular mechanisms underlying differences in beak morphology likely involve interactions among multiple embryonic populations. We exchanged neural crest cells destined to participate in beak morphogenesis between two anatomically distinct species. Quail neural crest cells produced quail beaks in duck hosts and duck neural crest produced duck bills in quail hosts. These transformations involved morphological changes to non-neural crest host beak tissues. To achieve these changes, donor neural crest cells executed autonomous molecular programs and regulated gene expression in adjacent host tissues. Thus, neural crest cells are a source of molecular information that generates interspecific variation in beak morphology.

In *On the Origin of Species*, Darwin skillfully argued that natural selection governs adult beak morphology, but he was hard put to explain how features of different taxa could arise during embryogenesis. After measuring full-grown beaks of pigeon breeds, which varied “extraordinarily in length and form,” and comparing them with those of newly hatched birds, Darwin

found that beak proportions of some breeds were already quite distinct in the young. He concluded, “each successive modification, or most of them, may have appeared at an extremely early period . . . from causes of which we are wholly ignorant” (1). Ever since Darwin’s studies, the role of natural selection in beak evolution has been well substantiated, but the developmental basis for interspecific variation has remained elusive.

Here, we investigate the cellular and molecular origins of beak morphology. Beaks among groups of birds are astonishingly variable, and yet at early embryonic stages they all arise from

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