

INTRODUCTION

Nutrient limitation (especially nitrogen [N] and phosphorus[P]) is an important driver in the development of various aquatic and terrestrial ecosystems. However, studies on nutrient limitation of the development of restored ecosystems are still few.

In the Hole-in-the-Donut (HID) area of the Florida Everglades, historic farming elevated P levels in soils, leading to the invasion of Brazilian pepper (*Schinus terebinthifolius*). To restore these wetlands, soil was removed to allow primary succession to revegetate the site. During this process, N- and P-limitation would occur in different sites and vary with the season and elevation.

The objective of this study is to assess

whether and how patterns of nutrient limitation would 1) differ between restored and reference wetlands, and 2) vary with the season and elevation (hydrology).

METHODS

Surface soils and periphyton were sampled from restored (2000-restored, 2003-restored) and reference wetlands in the HID of the Everglades;

Five transects along a elevation gradient from 0.5m to 1.0m with the interval of 0.1m; A and B were lumped as high elevation sites and D and E as low elevation sites (Fig. 1);

Sampling time: October, 2009 (wet season); February, 2010 (dry season);

Parameters for soil and periphyton measured: TN, TC, TP, microbial biomass C, N, P;

Enzymes measurement (see Table 1).

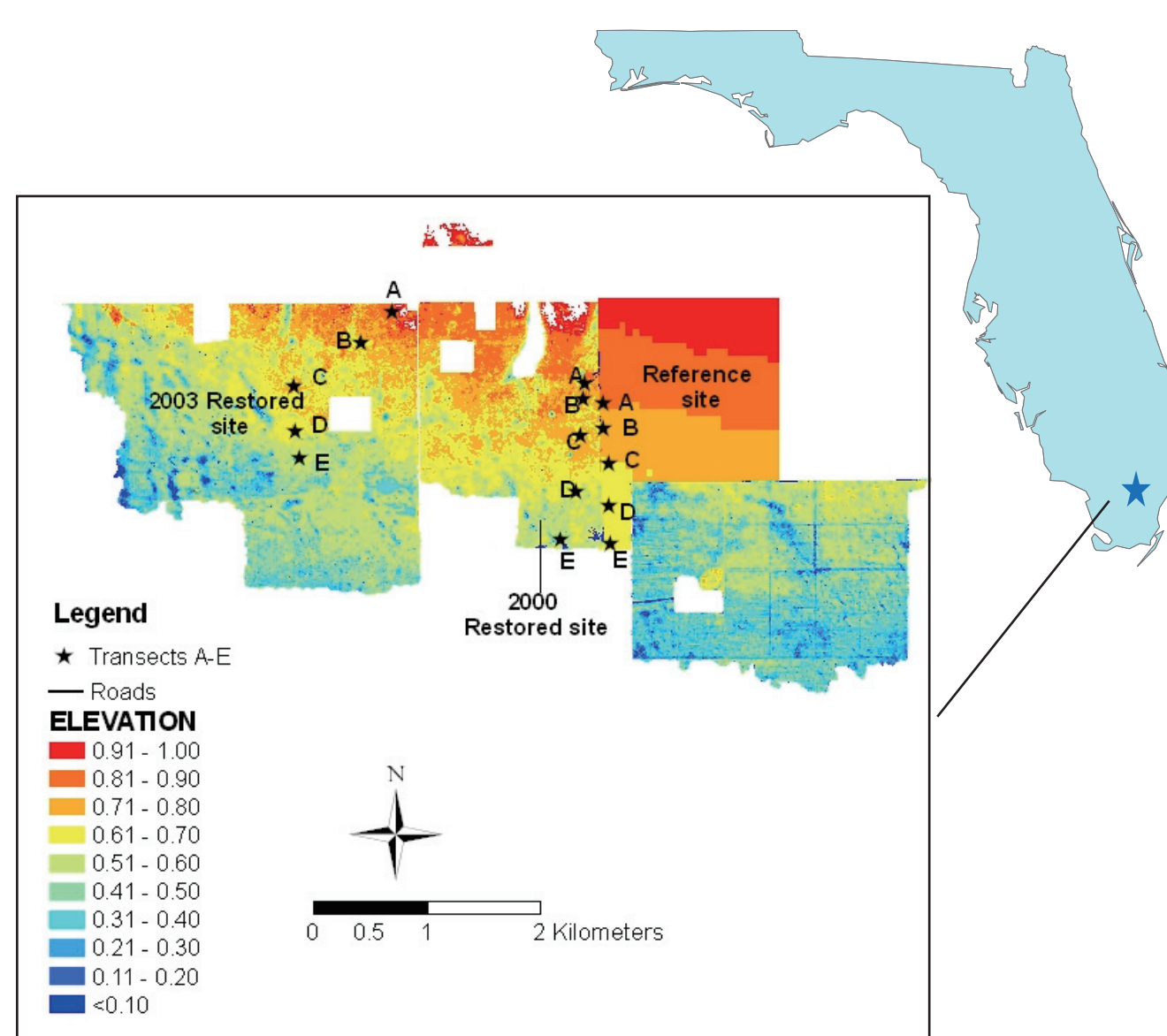


Fig.1 Sampling locations in the HID of the Everglades

Table 1 Methods for enzyme measurement

Parameters	Methods	Instrument
Alkaline phosphatase (Pase)	Substrate: umbelliferyl-substrates (MUF-substrates)	Bio-Tek Model FL600 fluorometric plate reader
Leucine aminopeptidase (LAP)	Substrate: leucine 7-amido-4-methyl coumarin (AMC)	At excitation of 350nm and emission of 450nm
N-acetyl-β-D-glucosaminidase (NAG)	Substrate: MUF-substrates	
Nitrogenase (N ₂ fixation)	Acetylene (C ₂ H ₂) reduction (AR) assay	Shimadzu GC-8A gas chromatograph

RESULTS

TN/TP ratios in restored and reference wetlands

For both the wet and the dry season, TN/TP molar ratio of soil and periphyton in the reference site were much higher than those in the two restored sites ($P < 0.001$; Fig. 2)

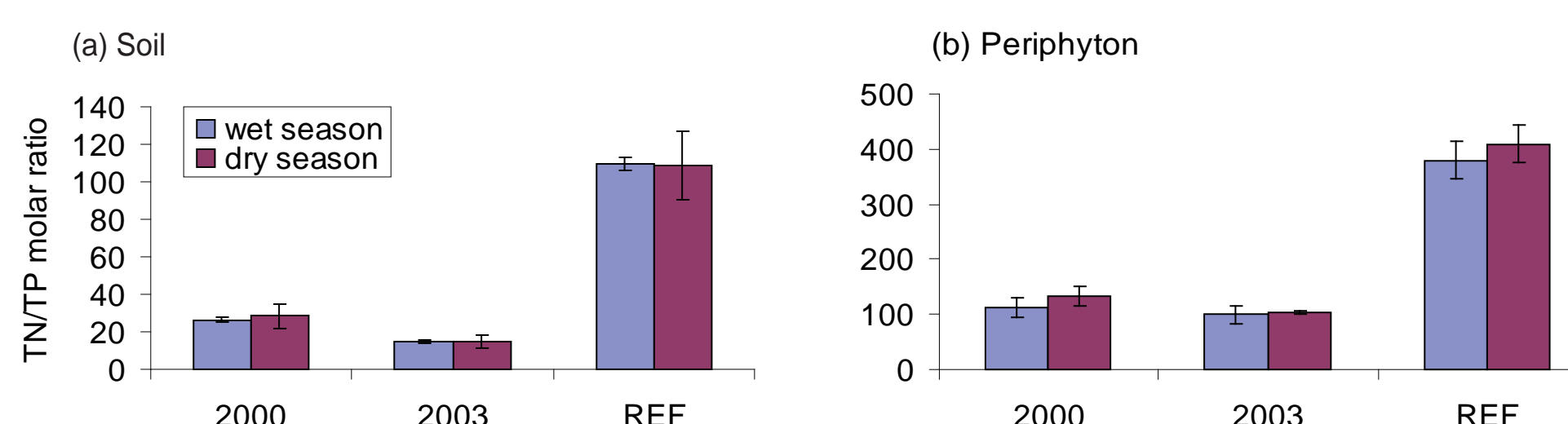


Fig.2 TN/TP molar ratio of soil and periphyton in the 2000-restored, 2003-restored and reference sites in the dry and wet season (mean±SE, n=15)

Enzymes activities in restored and reference wetlands

Higher N-related enzyme (i.e., NAG, LAP and nitrogenase) activities were observed in the restored sites in both the dry and wet seasons. In contrast, Pase activity was higher in reference sites than restored sites in both seasons. Enzyme activities were much higher in the wet season than in the dry season (Fig. 3).

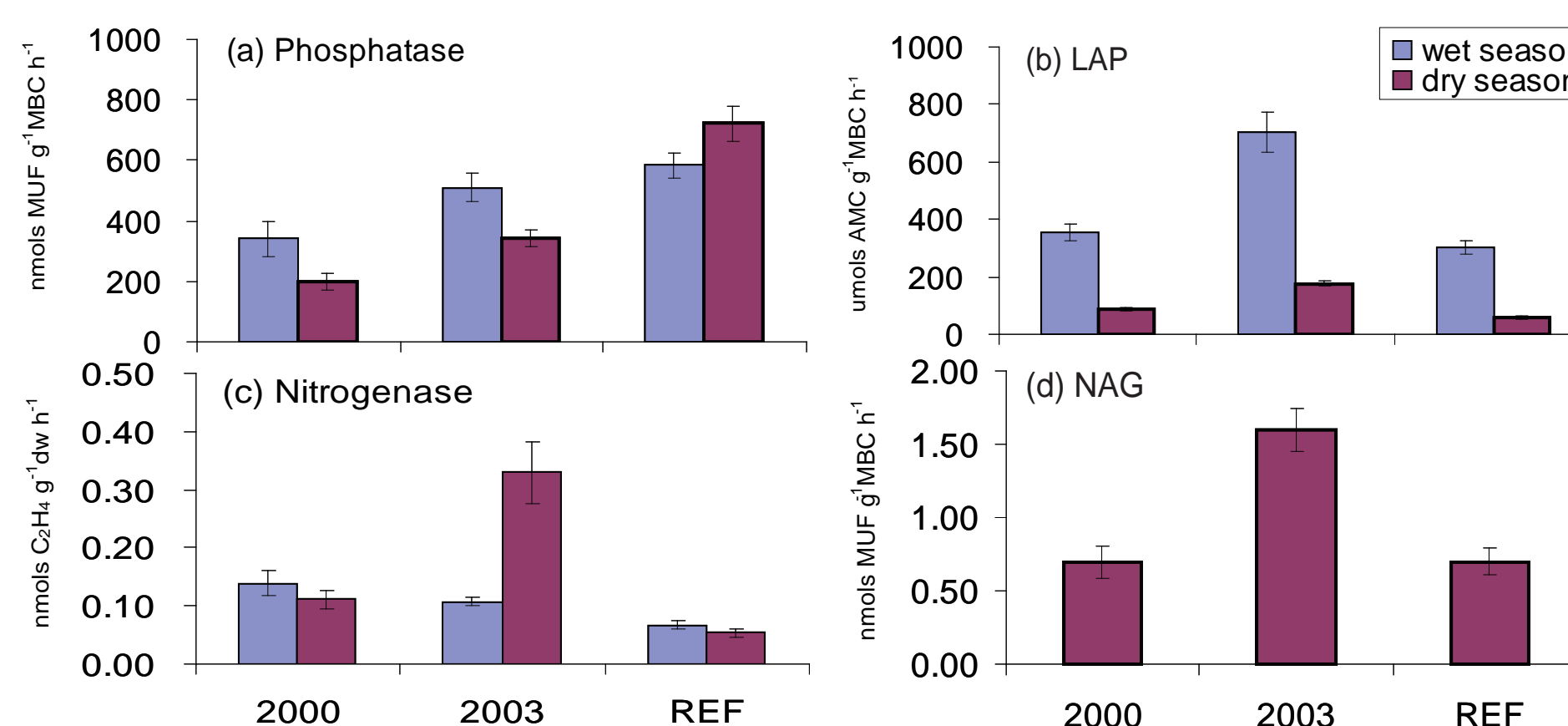


Fig. 3 Phosphatase (a), LAP (b), nitrogenase (c) and NAG (d) activities based on MBC and N₂ fixation of periphyton in the three sites in wet and dry season (mean±SE, n=15)

The role of elevation

The effect of elevation on enzyme activities (i.e.,LAP, NAG, Pase and nitrogenase) appeared to vary depending on the season and site restoration history. Higher enzyme activities (i.e.,LAP, NAG,Pase) were observed generally in low elevations than in high elevations, especially in the wet season. Nitrogenase activity, in contrast, was found higher in the high elevation site in the dry season (Fig. 4).

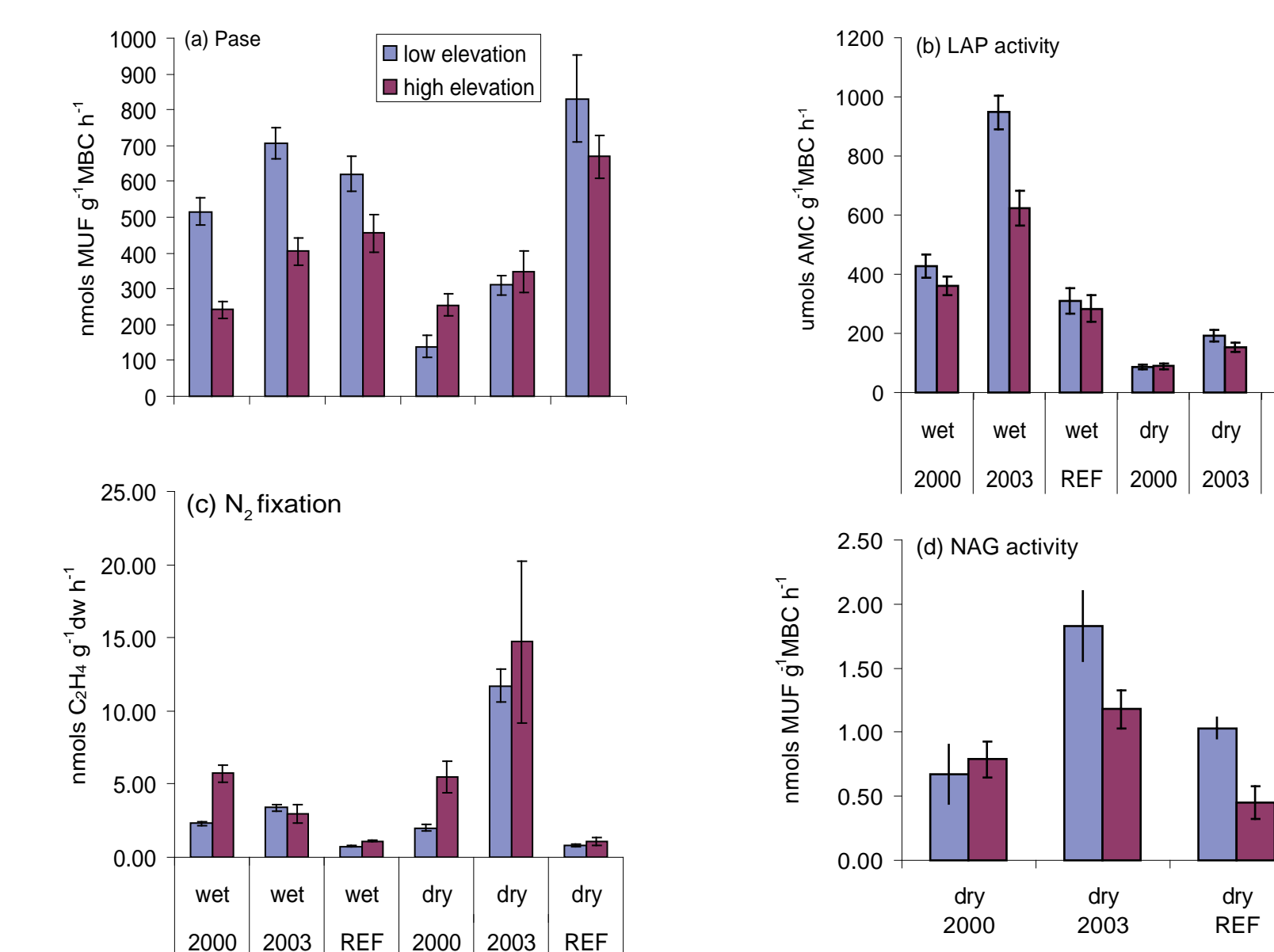


Fig.4 The effect of elevation on enzyme activities in different sites and seasons (mean±SE, n=6)

Relationship between enzymes and TN/TP ratio

LAP and nitrogenase activities were negatively correlated with TN/TP of soil and periphyton whereas Pase was positively correlated with TN/TP, regardless of the wet or dry season (Fig. 5).

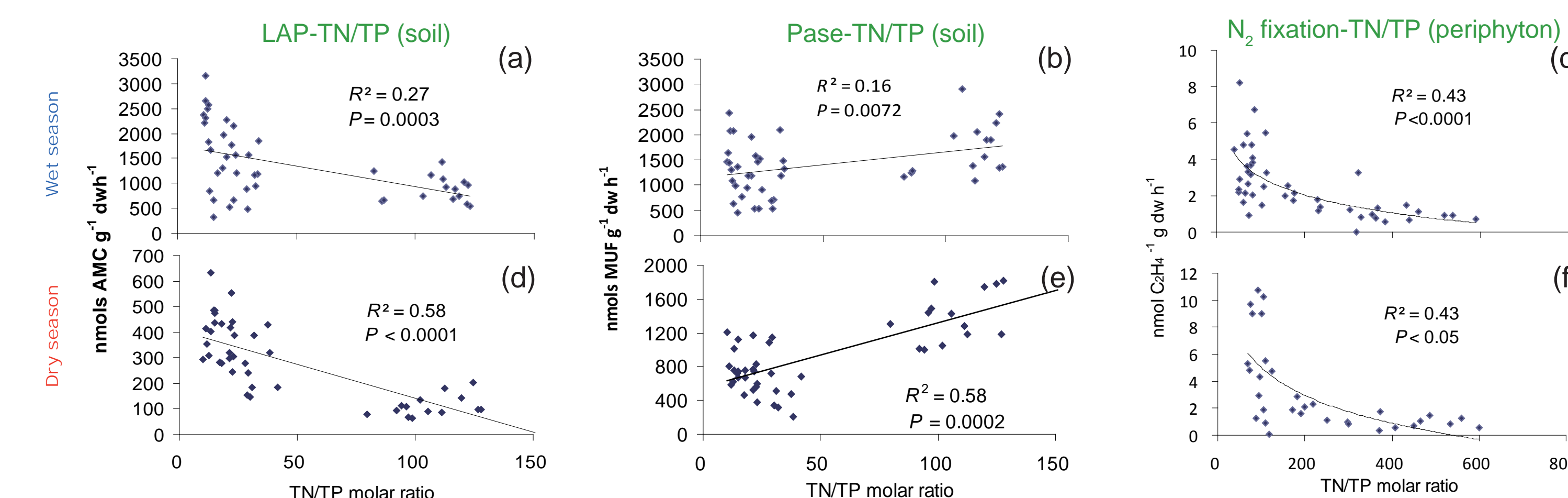


Fig.5 Relationship between enzyme activities and TN/TP molar ratio in soil (a, b, d and e) and periphyton (c and f). All sample sizes are 45.

CONCLUSIONS

Our results reported here indicate that

- (1) Younger restored wetlands could be more N-limited, while the reference site could be more P-limited;
- (2) The effect of elevation on nutrient limitation may vary depending on the season and site restoration history, suggesting environmental factors such as season and hydrology could influence availability of nutrients; and
- (3) TN/TP ratio and enzyme activities are good indicators of nutrient limitation.

