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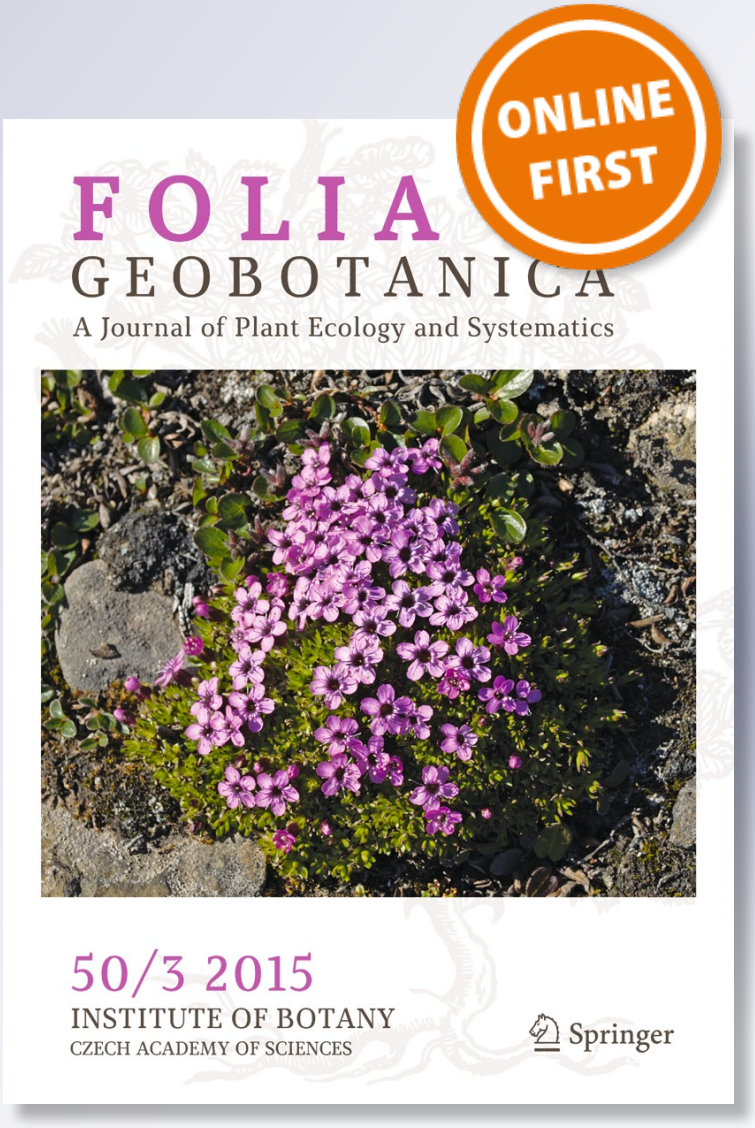


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# Phenolic content and growth of wetland macrophytes: Is the allocation to secondary compounds driven by nutrient availability?

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**Abstract** This study compares soluble phenolics and lignin content in two wetland macrophytes with contrasting life strategies grown under a varying nutrient supply in the field and in a greenhouse experiment. The differences are explained in terms of the protein competition model (PCM) hypothesis relating changes in secondary metabolites to changing nutrient limitation. The two study species, *Eleocharis cellulosa* (EC) and *Typha domingensis* (TD), are both widespread in tropical and subtropical freshwater and brackish marshes of the New World, and are often found in P-limited rather than N-limited conditions. TD is a fast-growing competitor with large nutrient requirements. EC is a stress tolerator, quite well adapted to growth in nutrient-limiting environments. In both species, the concentration of phenolics was negatively correlated with increasing growth (due to increasing nutrient levels). This is in agreement with the PCM hypothesis, which predicts an increase in phenolic synthesis when protein synthesis (and consequently growth) is low due to limited resource availability. An interesting difference was found in the correlation between tissue nutrients and phenolics. TD from both the field and the greenhouse showed a negative correlation between tissue P and phenolics, while EC displayed a significant negative correlation between tissue N and phenolics. EC is adapted to low P, and increased tissue P content represents luxury consumption (uptake of P for storage) which is not reflected in increased growth and thus is not correlated with phenolics. These are the first steps in elucidating the relationship among nutrient availability, growth and phenolic content in two important primary producers of tropical and subtropical marshes.

**Keywords** lignin · phenolics · macrophytes · *Typha domingensis* · *Eleocharis cellulosa*

## Introduction

Wetland and aquatic ecosystems worldwide are impacted by human-mediated increases in nutrient input (Downing et al. 1999; Schindler 2012). Yet many aspects of this impact are poorly understood. While growth responses leading to changes in community structure have been widely studied (Aerts and Berendse 1988; Craft et al. 1995; Miao and Sklar 1998; Rejmánková et al 2008), far less is known about changes in plant secondary metabolites following increased input of nutrients. They may have important consequences for ecological interactions and processes such as herbivory and decomposition (Aerts and de Caluwe 1997; Peñuelas and Estiarte 1998; Hättenschwiler and Vitousek 2000; Wong et al 2010; Kagata and Ohgushi 2011).

The primary goal of this study was to compare growth, phenolics and lignin content in two dominant

To the memory of Dr Dagmar Dykyjová – my great role model and mentor

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marsh species with contrasting life strategies and to discuss the differences in terms of existing hypotheses relating changes in secondary metabolites to changing nutrient limitation.

Phenolics are among the most widely distributed classes of secondary metabolites present in plants (Waterman and Mole 1994; Harborne 1997; Bezemer et al. 2000). Following Waterman and Mole (1994), I differentiate throughout the text between 'phenolics', meaning total soluble phenolics, and 'lignin', which belongs to a general group of phenolic substances but because of its insolubility and consequent biochemical inertness deserves separate treatment. Both may account for several percent of plant dry mass, thus representing an important energetic investment. Their levels are partly under genetic control and partly determined by environmental conditions (Koricheva et al. 1998; Peñuelas and Estiarte 1998; Close and McArthur 2002). There is a close connection between phenolics and proteins because phenylpropanoid-derived secondary metabolites are synthesized in the shikimate pathway and share a common precursor, phenylalanine, with protein synthesis. Phenolics serve many important functions in plants, such as structural support, herbivore defence and UV screening (Jones and Hartley 1999).

Since the 1950s, the general belief among ecologists has been that the primary role of secondary metabolites is to provide defence against herbivory. This led to the formulation of numerous hypotheses explaining the formation, distribution and role of various secondary metabolites. Several theories have been provided for the explanation of changes of secondary metabolites with changing environmental conditions (e.g. shading level, nitrogen availability, atmospheric $CO_2$). For a detailed review, see Stamp (2003), but briefly:

(1) The *carbon-nutrient balance hypothesis* (CNB, Bryant et al. 1983): According to the CNB hypothesis, the availability of carbon and nutrients in the environment determines the amount and kind of chemicals that a plant allocates to defence vs growth, and improved nutrient balance is predicted to result in decreased production of phenolic compounds.

(2) The *growth differentiation balance hypothesis* (Herms et Mattson 1992): According to this hypothesis, growth is largely limited by water and nutrients whereas differentiation depends mainly on available carbohydrates. Therefore, production of carbon-based secondary compounds dominates when factors other than photosynthate supply are suboptimal for growth (e.g. under nutrient limitation).

(3) The *protein competition model* (PCM, Jones and Hartley 1999) states that since phenolics have a common precursor (phenylalanine, PHE) with proteins, there is a trade-off between protein and phenolic synthesis. When growth is not limited by nutrients, more PHE will be used for protein synthesis and, consequently, growth. If nutrients become limiting, protein synthesis slows down and more PHE is used for phenolic synthesis.

(4) The *induced defence hypothesis* assumes that the phenolic content is regulated by the herbivore-plant interaction (Karban and Myers 1989).

These hypotheses, specifically (1) to (3), assume some kind of trade-off between allocation to growth vs carbon-based secondary metabolite production determined by nutrient availability, and they are to some degree complementary. Contrasting results have been reported on how successful these hypotheses are in predicting the concentration of carbon based secondary compounds in different plant species/types (Koricheva et al 1998; Hamilton et al. 2001). Mixed results are most likely due to large variation in compounds and environmental conditions. Additionally, interpretation of results based on concentrations of secondary metabolites rather than their content can lead to erroneous conclusions (Koricheva 1999; Hol et al. 2003). Because nitrogen is commonly the limiting nutrient in temperate terrestrial communities as well as in marine macroalgae (Pavia et al. 1999), the response of secondary metabolites to changes in N availability has been studied more often, while less information is available on phosphorus. It is also assumed that N will have a greater influence on the production of phenolic defensive compounds than P because N limitation reduces protein production and thus competition for phenylalanine, a precursor of many phenolic compounds (Wright et al 2010).

In this study, I choose two wetland plant species, *Eleocharis cellulosa* Torrey (EC) and *Typha domingensis* Pers. (TD), both widespread in tropical and subtropical freshwater and brackish marshes of the New World, often in P-limited rather than N-limited conditions. These species have distinctly different life strategies: TD is a fast-growing competitor with large nutrient requirements. EC is a stress tolerator, quite well adapted to growth in nutrient-limiting environments.

Their growth and response to nutrient increase have been well described (Craft et al 1995; Rejmánková et al. 1996; Richardson et al. 1999; Rejmánková 2001; Rejmánková et al. 2008). My main question was: How do growth and phenolic compounds of these two species respond to different levels of nitrogen and phosphorus? A survey of phenolic and lignin content in plants from marshes with different nutrient status was conducted first. Phenolics and lignin concentration, soil and plant tissue nutrient levels, and plant growth were measured. Since field data indicated a relationship among soil nutrients, plant growth and phenolics, a greenhouse experiment was conducted. Both species were grown in combinations of three nitrogen and three phosphorus levels, and the same variables as in the field were measured. I expected to find: (1) a negative relationship between phenolic concentration and growth; (2) a more significant growth response to increased nutrient input in TD (fast growing strong competitor) than EC (slow growing stress tolerator); and (3) higher concentrations of phenolics in EC than in TD under all nutrient treatments.

## Material and Methods

### Field sampling

Plant samples were collected from 24 marshes in northern Belize. For a detailed description of these wetlands, see Rejmánková (2001). Soil samples and plant tissue samples were collected in representative monospecific stands of *Typha domingensis*, *Eleocharis cellulosa* and *Cladium jamaicense*. Only mature leaves (TD) or stems (EC) without any signs of senescence were collected to assure the maximum potential homogeneity of plant material being compared.

### Greenhouse experiment (GH)

Plants were propagated from rhizomes collected in the Buena Vista Marsh, northern Belize. They were grown in 2-L pots filled with sand and placed in large trays with DI water (water level about 1–2 cm above the sand surface); pH ranged from 6 to 6.5. Microelements, Fe and $MgSO_4$, were added to the trays in concentrations corresponding to 0.25 Hoagland nutrient solution. Nitrogen and phosphorus were added to individual pots by injecting the solution of $Ca(NO_3)_2$ and $KH_2PO_4$ in the following concentrations:

| Nitrogen | Phosphorus |
|---|---|
| Low (LN) 1 mg N/pot (= 0.5 mg/L) | Low (LP) 0.1 mg P/pot (= 0.05 mg/L) |
| Medium (MN) 10 mg N/pot (= 5 mg/L) | Medium (MP) 1.0 mg P/pot (= 0.5 mg/L) |
| High (HN) 100 mg N/pot (= 50 mg/L) | High (HP) 10 mg P/pot (= 5 mg/L) |

The nutrients were added four times during the duration of the experiment (46 days). The full factorial design resulted in nine combinations, LNLP, LNMP, LNHP, MNLP, MNMP, MNHP, HNLP, HNMP and HNHP, each in four replicates for each species. At the time of harvest, subsamples of live leaf/stem tissue were immediately freeze-dried for phenolic and lignin analyses. The remaining aboveground tissue, rhizomes and roots were dried, weighed and analysed for N and C content on the Carlo-Erba series 5000 CHN-S analyser. Total phosphorus was analysed with ICP-AES (Inductively Coupled Plasma spectroscopy, Thermo Jarrell Ash Corporation, model Atom Scan 25) after microwave acid digestion (Sah and Miller, 1992).

### Growth

Growth was expressed as cumulative shoot (EC) or leaf (TD) length. In Belize, all EC shoots per 20 × 20 cm were counted and measured. For TD, the lengths of all leaves per 50 × 50 cm were measured. In the GH experiment, total shoot/leaf length was measured at 10 days intervals. Relative growth rate (RGR) of leaves was calculated and at the end of the experiment, and dry weights were determined for separated shoots/leaves, roots and rhizomes. Cumulative leaf/shoot length was well correlated with total biomass and RGR (TD: $R^2 = 0.98$; $P < 0.0001$; EC: $R^2 = 0.73$; $P = 0.003$) and was selected as the response variable to represent growth.

### Phenolics

Samples for phenolics and lignin from the GH experiment were freeze-dried immediately following sampling and finely ground in a ball-mill. Samples from the field were stored and transported frozen and then freeze-dried and ground. The ground material (0.05 g) was extracted

twice with 100 % methanol at room temperature. The extraction method was chosen as the one giving maximum yield after initial trials of several extraction techniques listed by Waterman and Mole (1994). The total soluble phenolic content in the extracts was determined colourimetrically using the Folin-Ciocalteau reagent (SIGMA F9252) and p-coumaric acid as a standard (Waterman and Mole 1994). Lignin was determined by the method of Iiyama and Wallis (1989).

### Data analysis

The effects of nitrogen and phosphorus on growth, soluble tissue phenolics and lignin content in plants from the GH experiment were evaluated by a two-way ANOVA. The effects of sediment nitrogen and phosphorus on plant growth and concentrations of secondary compounds were evaluated by multiple regressions. Response variables were averaged for each treatment, and regressions were calculated using the mean values. The relationship among plant tissue nutrients, soil nutrients, growth and soluble phenolic compounds was expressed in an arrow scheme (Fig. 1) because total $n$ was too small to perform a complete path analysis. Simple correlation coefficients were used to indicate the level of significance.

## Results

Both phenolic and lignin concentration of field-collected TD plants was about two times higher (phenolics: 2.2 to 4.6 %; lignin: 1.9–3.8 %) than in EC plants (phenolics: 0.7 to 2.3 %; lignin: 1.2–2.8 %). Both species contained a relatively similar proportion of the two phenolic classes (Table 1), and the two classes were tightly correlated ($R^2$ = 0.72; $P$ = 0.001). There were no differences in tissue N content and soil N between the two species, but tissue P was higher in TD, and this species also occurred more often in marshes with higher sediment P content (Table 1).

The soluble phenolic concentration of the greenhouse grown plants ranged from 8.7 to 13.6 % and from 1.6 to 5.3 % in TD and EC, respectively. The lignin concentration ranged from 2.6 to 5.1 % and from 2.3 to 2.9 % in TD and EC, respectively. Lignin in field samples was in a range similar to greenhouse samples: 1.9 to 3.8 % and 1.2 to 2.8 % for TD and EC, respectively. Lignin and phenolic concentrations were closely positively correlated in TD samples ($R^2$ = 0.83; $P$ = 0.0007), but there was no correlation between these components in EC samples.

The results of multiple regressions for the field samples show that all response variables in TD were significantly correlated with the amount of phosphorus in sediments, while nitrogen concentration did not have any effect (Table 2). Similarly for EC, cumulative shoot length and phenolic concentration were correlated with phosphorus in sediment, and nitrogen did not show any effect, but there was no correlation between lignin concentration of EC and any of the soil nutrients (Table 2).

In the greenhouse experiment, growth of both TD and EC responded strongly to increase in nitrogen. Growth of TD increased significantly with increasing phosphorus only under the highest level of nitrogen. Phosphorus growth response in EC was not significant (Table 3, Fig. 2). In TD the tissue concentration of phenolics and lignin significantly decreased with increasing phosphorus additions; increasing the concentration of nitrogen resulted in decreased concentrations of lignin but did not have a significant effect on phenolics (Table 3, Fig. 2). Concentrations of both nitrogen and phosphorus had a significant effect on phenolics in EC, but no effect on lignin (Table 3, Fig. 2).

The arrow scheme (Fig. 2) summarizes the relationships among soil nutrients, tissue nutrients, growth and phenolic concentration in the two species grown in the field in Belize and in the greenhouse. While individual relationships have been described above, the scheme allows for looking at them all in one picture and reinforces the fact that in each case, there was a negative correlation between growth and phenolic concentration. An interesting difference was found in the correlation between tissue nutrients and phenolics (Fig. 3). TD from both Belize and the greenhouse showed a negative correlation between tissue phosphorus and phenolics. Surprisingly, EC plants displayed a significant correlation between tissue nitrogen and phenolics. Both phenolics and lignin were negatively correlated with P in *Typha*. This was consistent for both greenhouse grown and Belize plants. There was no relationship between lignin and tissue nutrients in EC.

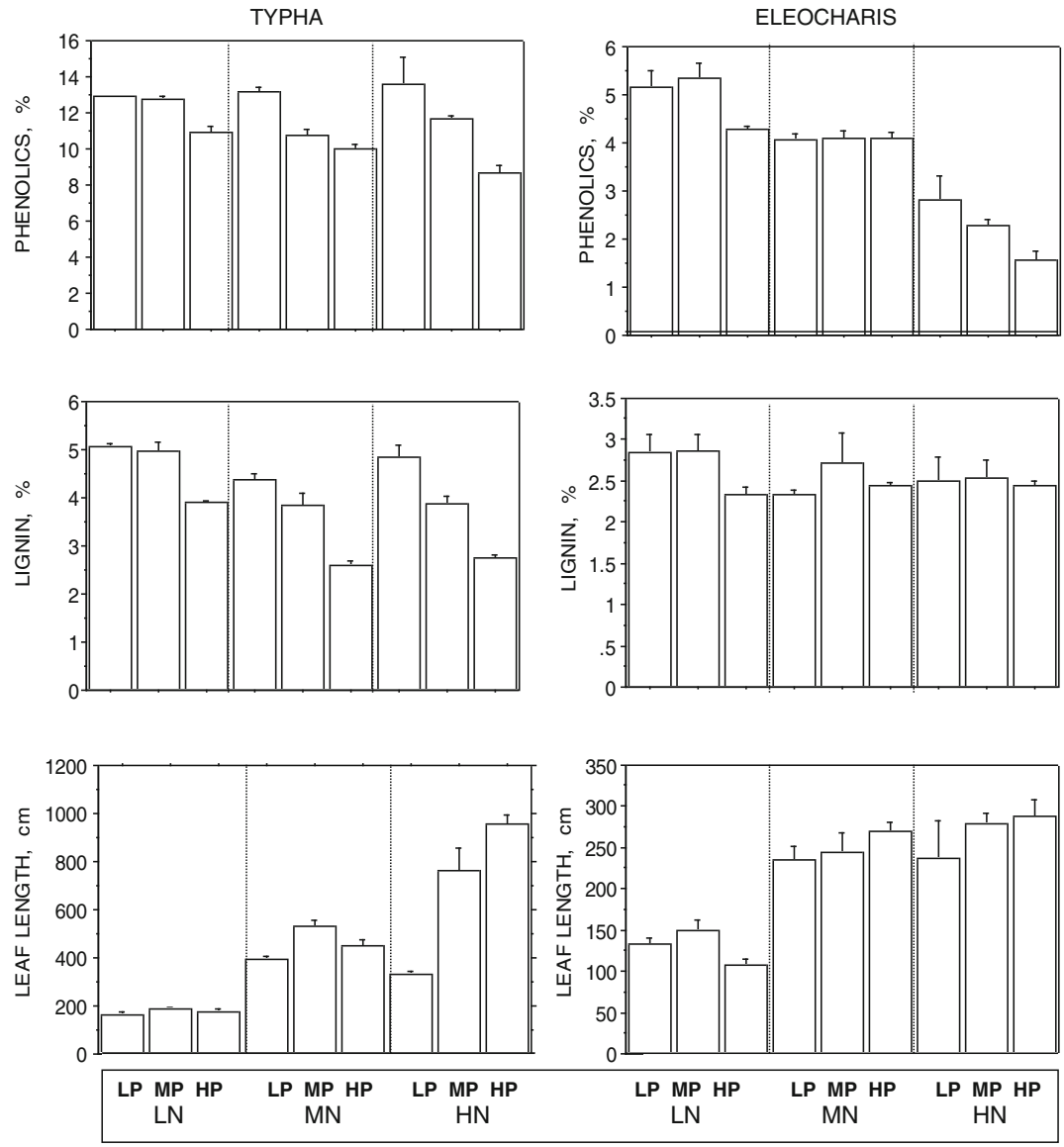


**Fig. 1** Percent of phenolics and lignin in dry tissue and cumulative leaf length per pot (y-axes) as a function of different combinations of nitrogen and phosphorus in the greenhouse experiment. LN – low nitrogen, MN – medium nitrogen, HN – high nitrogen, LP – low phosphorus, MP – medium phosphorus and HP – high phosphorus.

**Table 1** Mean values and ranges of soluble phenolics, lignin, tissue, and soil nitrogen and phosphorus in samples of *Typha domingensis* and *Eleocharis cellulosa* from Belizean marshes.

| | *n* | Phenolics [%] | Lignin [%] | Tissue N [%] | Tissue P [%] | Soil N [mg cm$^{-3}$] | Soil P [mg cm$^{-3}$] |
|---|---|---|---|---|---|---|---|
| Typha | 11 | 3.8 | 3.09 | 1.36 | 0.096 | 3.60 | 0.198 |
| | | (2.2–4.6) | (1.9–3.8) | (1.06–1.63) | (0.069–0.166) | (1.79–6.40) | (0.022–0.907) |
| Eleocharis | 13 | 1.6 | 1.58 | 1.16 | 0.050 | 3.21 | 0.055 |
| | | (0.7–2.3) | (1.15–2.82) | (0.75–1.90) | (0.038–0.076) | (1.11–4.52) | (0.019–0.102) |

**Table 2** Results of multiple regressions comparing the effects of nitrogen and phosphorus on growth (cumulative shoot/leaf length per plot), soluble phenolics and lignin concentration [%] in *Typha domingensis* (TD) and *Eleocharis cellulosa* (EC) from marshes of Belize. Significant correlations are indicated in **bold**.

| Factors | Standardized coefficients | *t* | *P* | Standardized coefficients | *t* | *P* | Standardized coefficients | *t* | *P* |
|---|---|---|---|---|---|---|---|---|---|
| | TD leaf length [m] | | | TD phenolics [%] | | | TD lignin [%] | | |
| N | 0.020 | 0.086 | 0.9334 | 0.054 | 0.226 | 0.8266 | 0.110 | 0.495 | 0.6340 |
| **P** | **0.823** | **3.603** | **0.0070** | **−0.845** | **−3.538** | **0.0076** | **−0.896** | **−4.020** | **0.0038** |
| Intercept | 32.883 | 5.644 | 0.0005 | 4.171 | 9.941 | < 0.0001 | 3.232 | 10.731 | < 0.0001 |
| | EC leaf length [cm] | | | EC phenolics [%] | | | EC lignin [%] | | |
| N | 0.213 | 0.925 | 0.3790 | 0.135 | 0.493 | 0.6337 | −0.278 | −0.847 | 0.4187 |
| **P** | **0.661** | **2.866** | **0.0186** | **−0.688** | **−2.533** | **0.0321** | 0.400 | 1.219 | 0.2539 |
| Intercept | −263.825 | −0.508 | 0.6233 | 2.043 | 4.845 | 0.0009 | 1.511 | 5.139 | 0.000 |

## Discussion

The response to the main question, whether the allocation to phenolic compounds is driven by nutrient availability, is affirmative, although differences exist among the two species in their response to increasing nitrogen vs phosphorus.

According to my predictions, both species, TD and EC, grew larger and denser in marshes with a higher concentration of P in the sediments, which is not surprising in a system that is generally P-limited (Rejmánková and Komárková 2000). In a similar wetland system, the Florida Everglades, a sedge, *Cladium jamaicense*, was reported to grow significantly better in P-enriched areas than in P-limited sites (Richardson et al 1999). The lack of a response to N found in the Belize dataset can be explained by the fact that P limitation overrides any potential effect of higher N content in sediments (Rejmánková and Snyder 2008). Contrary to the results from the field, in the greenhouse the growth of both species was strongly positively correlated with increasing levels of N, while there was less significant (TD) or no significant (EC) response to an increase in P. Why did the species in the greenhouse experiment respond differently? A direct comparison of the nutrient situation in marshes vs the greenhouse is difficult. Soil samples from the marshes were analysed for total N and P, the available forms of N and P were not measured. From previous experience we know that total N and P in those soils is about 200–600 times higher than the available forms (Rejmánková et al. 1995 and unpublished data). That would make the low level of N and P in the greenhouse experiment correspond to about one-third to one-half of the nutrient availability in marsh soils, while the medium and high N and P would represented about 5× and 50× higher availability, respectively. Apparently, even the low P addition was enough to prevent P limitation at low and medium N.

**Table 3** Results of two-way ANOVA comparing the effects of nitrogen and phosphorus on growth (cumulative leaf/shoot length per pot), soluble phenolics and lignin concentration [%] in *Typha domingensis* (TD) and *Eleocharis cellulosa* (EC) from the greenhouse experiment.

| Factors | MS | *d.f.* | *F* | *P* | MS | *d.f.* | *F* | *P* | MS | *d.f.* | *F* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TD leaf length [cm] | | | | TD phenolics [%] | | | | TD lignin [%] | | | |
| N | **2.004** | **2** | **318.2** | **< 0.0001** | 3.022 | 2 | 2.5 | 0.1015 | **3.560** | **2** | **37.3** | **< 0.0001** |
| P | **0.273** | **2** | **43.4** | **< 0.0001** | **34.408** | **2** | **28.4** | **< 0.0001** | **8.802** | **2** | **92.1** | **< 0.0001** |
| N * P | **0.085** | **4** | **25.0** | **< 0.0001** | 3.138 | 4 | 2.6 | 0.0593 | 0.288 | 4 | 3.0 | 0.035 |
| | EC leaf length [cm] | | | | EC phenolics [%] | | | | EC lignin [%] | | | |
| N | **0.715** | **2** | **54.7** | **< 0.0001** | **31.707** | **2** | **82.1** | **< 0.0001** | 0.246 | 2 | 0.8 | 0.464 |
| P | 0.015 | 2 | 1.1 | 0.3214 | **2.702** | **2** | **6.9** | **0.0048** | 0.382 | 2 | 1.2 | 0.311 |
| N * P | 0.062 | 4 | 2.3 | 0.0765 | 1.877 | 4 | 2.4 | 0.0804 | 0.424 | 4 | 0.7 | 0.609 |

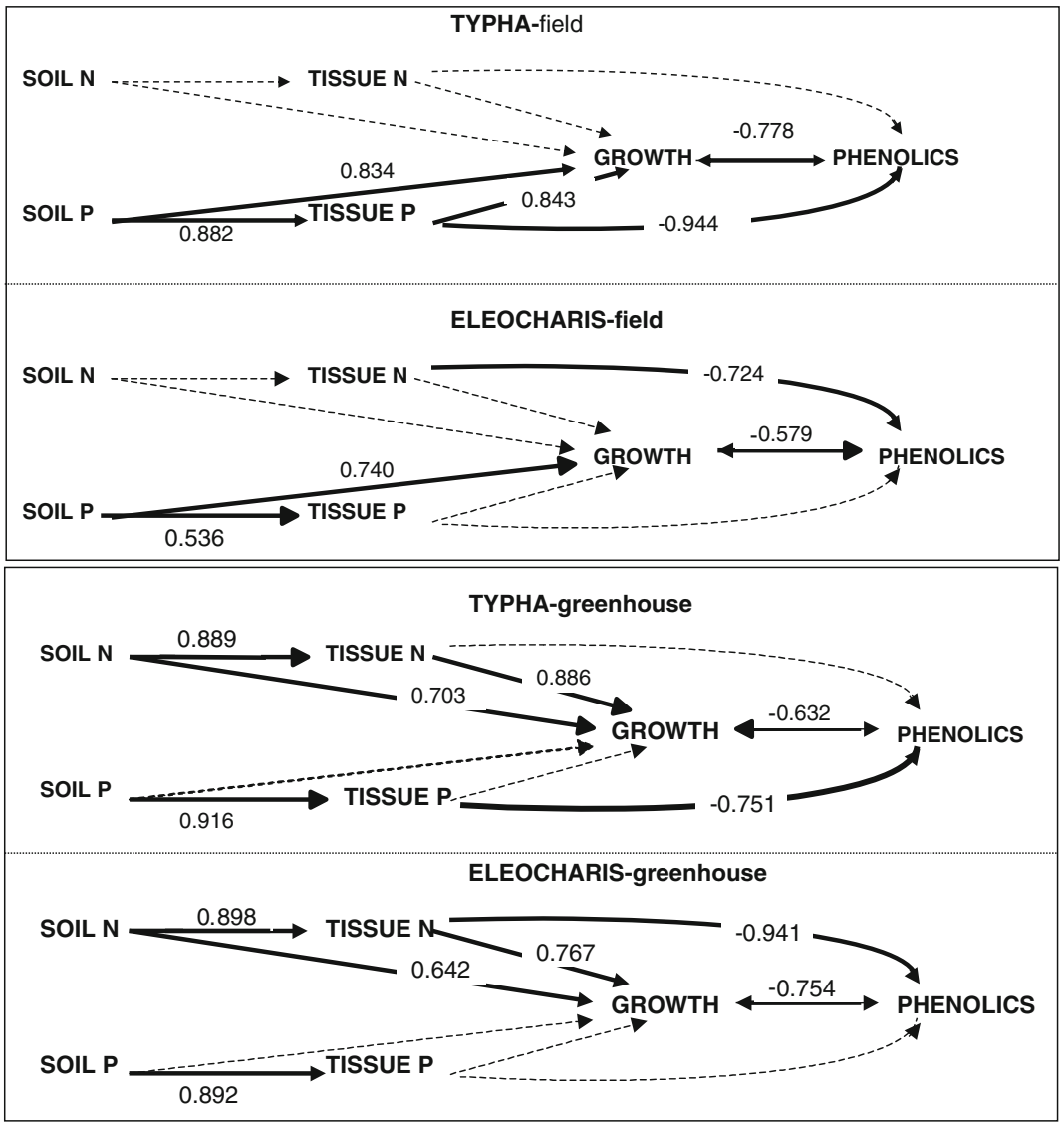


**Fig. 2** Relationships between soil and tissue N and P, growth, and phenolic content in *Typha domingensis* and *Eleocharis cellulosa* from Belize marshes and from the greenhouse experiment. Dotted lines indicate non-significant correlations; full lines show significant correlations ($P < 0.05$) with the numbers representing simple correlation coefficients.

The prediction of a negative relationship between phenolic concentration and growth was confirmed for both species from both environments (greenhouse and marshes). Similar findings were reported for *Cladium jamaicense* in the Florida Everglades (Richardson et al 1999). Plants growing in the P-enriched areas had much higher biomass while containing only half of the concentration of phenolics found in the plants from P-limited sites. These findings agree with the protein competition model (PCM) hypothesis predicting an increase in phenolic synthesis when protein synthesis (and consequently growth) is low due to limited resource availability. Contrasting results were reported by Feller et al. (1999), who found increased levels of phenolics in mangrove leaves in response to P enrichment. Similarly, treatments that strongly enhanced growth (N, P addition) did not reduce the concentration of phenolics in *Populus balsamifera* in Alaska (Reichardt et al. 1991). Wright et al. (2010) comparing foliar N and P concentrations and phenolic compounds in forest trees from P-rich alluvial soils vs P-limited marine terraces found differences in foliar P but not in N and phenolics. They

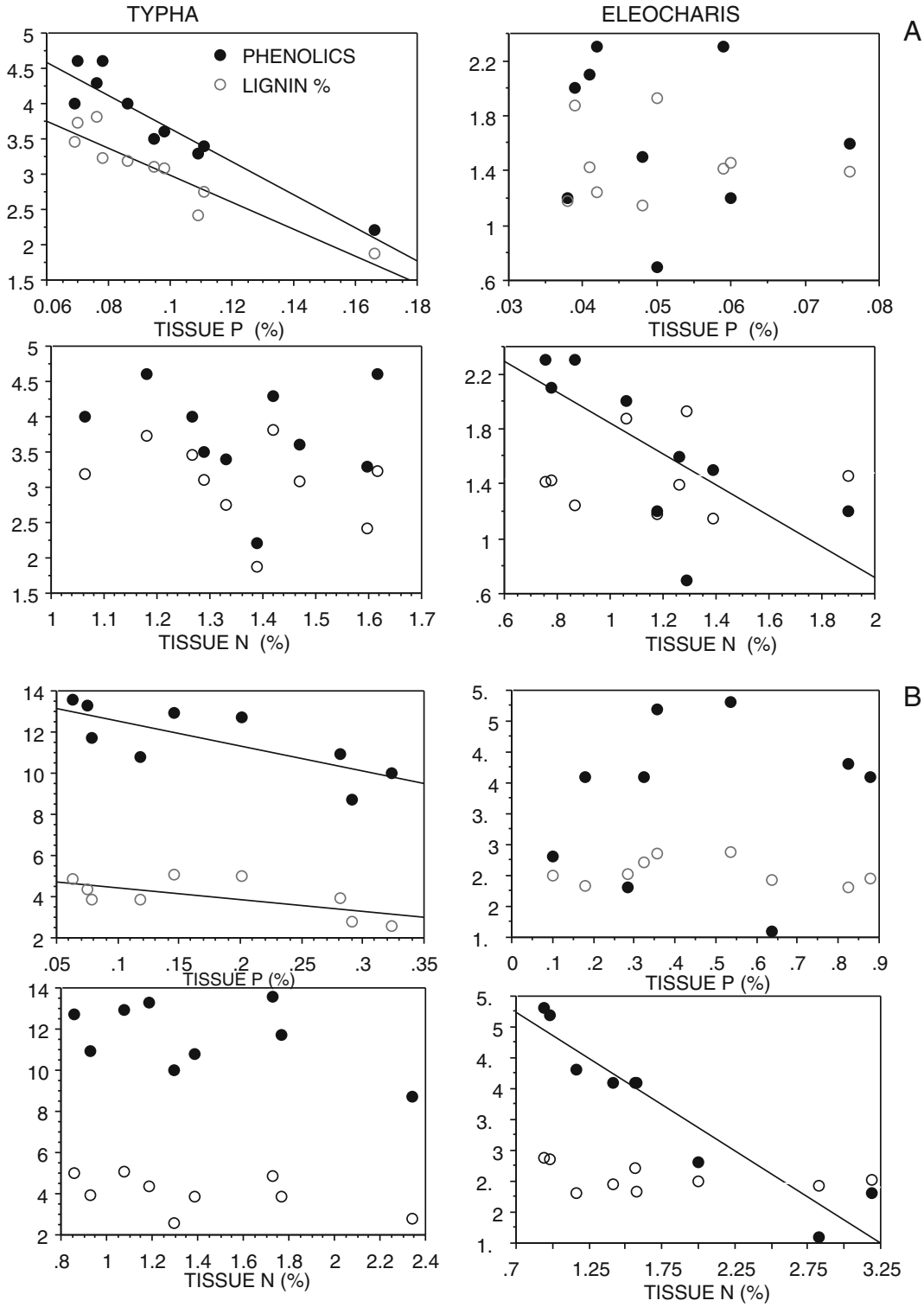


**Fig. 3** Relationship between phenolics, full circles, or lignin, empty circles, (y-axes, %), and nitrogen or phosphorus tissue concentration (x-axes). **A** – Belize marshes, **B** – greenhouse experiment. Regression lines are plotted for significant relationships only ($P < 0.05$).

interpreted these results as supporting the hypothesis (their extension of PCM) that N availability is a more important determinant of plant investment in phenolic defensive compounds than P availability. It is conceivable that while N availability is overall more important, in strongly P-limited environments (such as the marshes of Belize), it is P enrichment which determines changes in allocation to phenolics. Generally, these contrasting results point to complexity of the whole secondary metabolites × growth issue. The problem is that contrary to the relatively rich information available on the secondary metabolites in woody plants (see the meta-analysis by Koricheva et al 1998), very little is available on herbaceous vegetation (Table 4).

Senescent biomass of wetland macrophytes is the ultimate source of carbon for sediment microorganisms, and through leaching as dissolved organic matter (DOM) it can reach and impact adjacent water bodies. The difference in phenolic content of macrophyte litter can have important implications for processes such as decomposition (Snyder and Rejmánková 2015), yet despite its potential importance, data on phenolic and lignin concentrations in emergent macrophytes are very rare. Both phenolic and lignin values found in this study are low. They fall in the ranges reported for the Cyperaceae and Typhaceae (Table 4), which are lower than values presented for floating-leaved and emergent macrophytes by Smolders et al. (2000), and much lower than values reported for terrestrial plants, specifically trees (15–40 %, see Taylor et al 1989; Vitousek 1998; Novaes et al 2010). The only exceptions were higher phenolic values for TD from the greenhouse study. A potential reason for this discrepancy could be the different sample treatment. Greenhouse samples were frozen in liquid nitrogen immediately after harvest, while samples from Belize had to be first transported to the field lab, frozen, transported to Davis and only then freeze-dried. As stated by Waterman and Mole (1994), chemical changes in the material can be encountered during the collection and transportation of samples.

Species from nutrient-poor sites have been generally characterized by slow growth and a high concentration of secondary compounds (Aerts and Chapin 2000), which is why I predicted that EC would have a higher

**Table 4** Comparison of phenolics and lignin content in emergent macrophytes. Where two methods are given, the first applies to phenolics and the second to lignin.

| Species | Phenolics [%] | Lignin [%] | Method | Reference |
|---|---|---|---|---|
| *Typha angustifolia* | 2.8 | N/A | Folin-Ciocalteau | Bolser et al. 1998 |
| *Typha latifolia* | 2.5 | N/A | Folin-Ciocalteau | Bärlocher and Biddiscombe 1996 |
| *Typha latifolia* | N/A | 3.2 | fiber assay | Lacki et al. 1990 |
| *Typha domingensis* | 0.7 | N/A | in leachate | Maie et al. 2006 |
| *Lythrum salicaria* | 6.5 | N/A | Folin-Ciocalteau | Bärlocher and Biddiscombe 1996 |
| *Eleocharis dulcis* | N/A | 1.22 | HPLC | Parr et al. 1996 |
| *Eleocharis* sp. | 0.2 | N/A | in leachate | Maie et al. 2006 |
| *Carex heliophyla* | 1−1.3 | N/A | Prussian blue | Mole and Joern 1993 |
| *Cyperus rotundus* | 1.4 | N/A | Folin-Ciocalteau | Quayyum et al. 2000 |
| *Carex* spp. | 1.5−4 | 3.5−6.5 | Folin-Ciocalteau acetyl bromide | Aerts and De Caluwe 1997 |
| *Scirpus acutus* | N/A | 3.8 | fiber assay | Godshalk and Wetzel 1978 |
| *Cladium jamaicense* | 0.9−1.7 | N/A | Folin-Ciocalteau | Richardson et al 1999 |
| Submergent | 2.9 | N/A | Hagerman and Butler | Smolders et al. 2000 |
| Floating | 8.5 | N/A | Hagerman and Butler | Smolders et al. 2000 |
| Emergent | 7.4 | N/A | Hagerman and Butler | Smolders et al. 2000 |
| *Eleocharis cellulosa* (Belize) | 0.7−2.3 | 1.2–2.8 | Folin-Ciocalteau acetyl bromide | this paper |
| *Eleocharis cellulosa* (Greenhouse) | 1.6−5.3 | 2.3−2.8 | —"- | this paper |
| *Typha domingensis* (Belize) | 2.2−4.6 | 1.9−3.8 | —"- | this paper |
| *Typha domingensis* (Greenhouse) | 8.7−13.6 | 2.6−5.0 | —"- | this paper |
| *Cladium jamaicense* (Belize) | 4.8 | 3.9 | —"- | this paper |

concentration of phenolics and lignin than TD. EC is a species well adapted to growth in nutrient- (particularly P-) limited marshes. On the contrary, TD prefers more nutrient-rich sites and can grow very fast when not-limited by nutrients (Rejmánková et al 1996; Richardson et al. 1999). The results did not confirm my prediction. The phenolic and lignin content of EC was less than half of TD from both the greenhouse experiment and the marshes. In a related study, Maie et al (2006) found that litter of TD leached about 4× more phenolics than litter of *Eleocharis* sp. Apparently, the lower concentration of these compounds in EC as compared to TD is a result of genetic differences between the species.

Herbivory in Belizean marshes does not seem to be substantial. Apple snails (*Pomacea flagellata*) graze on both EC and TD, but due to relatively low snail densities, their impact is minor (Lege 2000). In addition to snails, there is occasional grazing by birds. Apple snails seem to prefer TD, which has a higher tissue nutrient content but apparently a higher phenolic content as well. Therefore herbivory does not seem to be the factor determining phenolic concentration. This is reinforced by the fact that the greenhouse-grown plants that grew completely without any herbivory contained more phenolics than marsh plants. If the formation of these secondary compounds were induced by herbivory, the plants grown in the greenhouse would have to have less phenolics. Thus my results indicate that phenolic formation in these two macrophytes is constitutive.

The interesting finding was the discrepancy between TD and EC when correlating their tissue N and P with phenolics. In TD, phenolic content was strongly negatively correlated with the tissue P concentration, while the relationship between tissue N and phenolic content was insignificant. This was found in both greenhouse-grown samples and in the samples from the field. On the contrary, in EC, phenolic content was significantly negatively correlated with tissue N in both greenhouse and field samples while there was no relationship between tissue P and phenolic content. This 'unresponsiveness' of EC phenolics to tissue P can be explained by the fact that EC is adapted to low P and increased tissue P content represents luxury consumption (uptake of P for storage) which is not reflected in increased growth. Luxury uptake has been known in plants from nutrient limited conditions that are exposed to an increase in the limiting nutrient (Aerts and Chapin 2000). On the other hand, increased tissue N in EC indicates improved growth, and thus negative correlation to phenolics. In TD, increased input of P leads to faster growth and, consequently, to lower phenolic synthesis.

Lignin seemed to be less responsive to nutrient changes, and its content was quite low, especially in EC. EC is much shorter plant apparently requiring less cell wall strength than tall TD. While there was no relationship between growth and lignin content in EC, lignin in TD was negatively correlated to growth. Similarly, negative correlation between lignin and growth has been reported by Novaes et al (2010).

## Conclusion

These are the first steps in elucidating the relationship between nutrient availability, growth and phenolic content in two important primary producers of tropical and subtropical marshes. In both species, the concentration of phenolics was negatively correlated with increasing growth (due to increasing available nutrients). Both lignin and phenolic content was relatively low and, contrary to the prediction, EC contained only about half of these compounds than the fast-growing TD. If the eutrophication of the Belizean marshes continues and they become dominated by TD, slower decomposition due to higher phenolic compounds will most probably result in a significant impact on sediment accretion.

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