Fire-mobilized nutrients from hydrophyte leaves favor differentially *Typha domingensis* seedling growth

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**A B S T R A C T**

In wetlands, altered disturbance regimes can change species composition and favor the expansion of invasive species. In Central Mexico, *Typha domingensis* expands after fires to colonize areas previously dominated by other natives, but the mechanisms are unknown. To explore if the invasion is facilitated by nutrient release after fire, we tested the effect of ash from *Schoenoplectus americanus* and *T. domingensis* plants on the growth of *T. domingensis* seedlings. *Schoenoplectus* ash had higher concentration of soluble phosphates (0.68 ± 0.23 mg/g) than *Typha* ash (0.36 ± 0.08 mg/g). At two months *Typha* seedlings were taller (48 ± 8 cm) in the treatment with *Schoenoplectus* ash added than in either the treatment with *Typha* ash (29 ± 7 cm) or in the control (25 ± 6 cm). Aboveground biomass (1.03 ± 0.42 g, 0.36 ± 0.17 g, 0.27 ± 0.11 g) and belowground biomass (0.65 ± 0.24 g, 0.32 ± 0.11 g, 0.27 ± 0.11 g) showed the same trends. *Typha* seedlings fertilized with *Schoenoplectus* ash had the lowest root:shoot ratios, but the largest rhizomes (dry weight 0.07 ± 0.03 g), followed by those fertilized with *Typha* ash (0.04 ± 0.01 g) and control plants (0.03 ± 0.01 g). These results show that *T. domingensis* responds to increased phosphate availability from ash of other species by growing larger and allocating more to storage tissues. We conclude that fire promotes the spread of *Typha* by facilitating seedling growth especially in areas dominated by *S. americanus*.

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1. Introduction

Proliferation of invasive species is the second greatest cause of biodiversity loss worldwide (Vitousek \textit{et al.}, 1997). In wetlands, an example of this general phenomenon is the tendency of species of *Typha* to increase to virtual total dominance, greatly reducing local diversity (Shih and Finkelson, 2008). Several studies suggest that this capacity to dominate may be due to the ability of *Typha* to exploit high nutrient levels (Rejmánková and Macek, 2007). In particular, it is the greater abundance of the macronutrients nitrogen and phosphorus that can increase photosynthetic rates and therefore productivity in this species, that leads to invasiveness (Woo and Zedler, 2002). This is a typical response of opportunistic plant invaders (Grime, 1979; Mack \textit{et al.}, 2000), that out-compete natives by being more productive in response to disturbance (Zedler and Kercher, 2004). As a result, where the nutrient levels of wetlands are high, such as in areas subject to runoff from agricultural fields (Davis, 1991; Koch and Reddy, 1993) or areas where fires have mineralized organic phosphorus (Smith and Newman, 2001), *Typha* spp. have been observed to expand to levels inimical to local diversity (Craft, 1995; Smith \textit{et al.}, 2001; Woo and Zedler, 2002; Levin \textit{et al.}, 2006; Galatowitsch \textit{et al.}, 1999; Zedler and Kercher, 2004).

*Typha domingensis* Pres. is characteristic of many freshwater wetlands in western Mexico (Rojas Moreno and Novelo Retana, 1995; Ramos Ventura and Novelo Retana, 1993). In our model system, the Mintzita springs and wetland system located to the southwest of the City of Morelia, state of Michoacán, Mexico (19°8′43″ N, 101°7′47″ W), a natural zonation occurs in which *T. domingensis* is dominant where the water is deeper while *Schoenoplectus americanus* dominates on the outer edge of the wetlands (Escutia-Lara \textit{et al.}, 2009a). This zonation has recently been disrupted after a series of fires. Beginning in 2005, some areas of the wetlands were burned by fires that spread from agricultural fields or that were started within the wetlands by land owners. Fire has had a differential effect on the dominant species. The distribution of *S. americanus* along the hydric gradient is not affected; but *T. domingensis* increased its cover by establishing from seeds, expanding into areas formerly dominated by *S. americanus*. As a result the initial zonation characteristic of these species has been lost (Escutia-Lara \textit{et al.}, 2009a). Since *Typha* spread by seed, and fire alter nutrient regimes, we have hypothesized that changes in distribution patterns in areas formerly dominated by other native
species are, at least partly, the result of ash enriching the soil with phosphorus, thus facilitating *Typha* establishment. Fire has been observed to have a variety of effects on wetlands depending on many factors that are only partly understood. In several types of wetlands fire has changed species composition (Johnson and Knapp, 1995; Kost and Steven, 2000; Escutia-Lara et al., 2009a) with the direction and degree of change apparently related to their historical natural disturbance regimes. Plant life history plays a role. In some cases annual species are favored (e.g., Warners, 1989) while in others the balance shifts to perennials (Bowles et al., 1996). This suggests that fire related responses of wetland plant communities are dependent on plant species composition and interactions with other factors. Indeed, fire also alters insect populations and interactions in wetland communities (Hochkirch and Adorf, 2007), primary production (Gabrey and Afton, 2001), and nutrient regimes (Laubhan, 1995). Furthermore, fire temperature and the nutrient content of ash (Quian et al., 2009a) and post-fire nutrient loss (Quian et al., 2009b) have been shown to affect wetland composition and dynamics.

In this study we focus on the direct effect of changes in nutrient availability resulting from ash deposition on the establishment and early growth of *T. domingensis*, a species favored by fire. Although this process has been studied in agricultural systems, little is known about it in natural wetland ecosystems (Baziar et al., 2009). We postulated that the spread of *Typha* may be due to the increased availability of nutrients, particularly phosphorus. To test this hypothesis, we conducted greenhouse trials to assess the response of *T. domingensis* seedlings to ash derived from the aerial parts of *S. americanus* and *T. domingensis* grown under different nutrient regimes.

2. Materials and methods

2.1. Plants for ash production

We set up 24 mesocosms with a capacity of 2101 (100 cm × 68 cm × 40 cm) in an outdoor facility at the Centro de Investigaciones en Ecosistemas (4.6 km east of the Mintzita wetlands), 12 for each species. We placed 9 pots of 51 (25 cm in height × 16 cm in diameter) in each mesocosm with each of the pots receiving one rhizome of either *S. americanus* or *T. domingensis*. The smallest possible rhizome size was used to reduce the effects of stored nutrients. For *T. domingensis* the size was ca. 15 cm long with one active bud and for *S. americanus* ca. 10 cm long with at least 3 active buds, because for this species high mortality occurred if fewer buds were present. The mesocosms were leveled and a constant water level was maintained with a float valve that fed each mesocosm with tap water (pH 7.2 ± 0.2; HCO3 < 200 ppm; Mg, Na, and Cl all below 20 mg/l).

Each of the pots in the mesocosms was fertilized with a different amount of phosphorus and nitrogen within the range observed in the field and previously tested under controlled conditions (Escutia-Lara et al., 2009b). Plants were grown under different N:P fertilization treatments to provide a more realistic range of growing conditions. We wanted to avoid creating artificially homogeneous conditions that might have led to low variability in the chemical composition of the resulting ash samples. The P was supplied in the form of KH2PO4 at three levels: 0.068 and 0.137 g, and control (none). N was applied as KNO3 in two concentrations: 0.15 and 0.30 g, and a control (none); in an orthogonal design for each species so as to yield 9 treatments of fertilizer regime with 12 replicate pots per treatment per species.

The fertilizer was dissolved in 15 ml of distilled water and applied on the surface of each pot. The treatment without added nutrients consisted solely of 15 ml of distilled water. Nutrients were supplied every fortnight during the six months spanning the growing season (May–October, 2008). The water in the mesocosms was replaced once a month. Six mesocosms were chosen at random every week and the water tested colorimetrically for phosphates and nitrates (Hach Phosphate Test Kit No. 2249-02 and Nitrate Test Kit No. 1416100, Hach Company, Colorado, USA). For all sampling dates phosphate and nitrate levels were close or below the detection limit of the tests.

After the growing season aerial biomass, which was mostly dry at the time, was harvested. To obtain the ash the biomass of all plants of the same treatment was combined and burned in a cylinder made of chicken wire to keep the leaves upright and in a density similar to that observed in the field. Ash was stored in sealed containers until use. During the experiment, 30 *S. americanus* plants and 10 *T. domingensis* plants were damaged by herbivores and other factors, these plants were not used for producing ash and were not included in the statistical analyses.

2.2. Ash fertilization experiment

*Typha* seedlings were obtained by planting seeds from 20 plants growing in the Mintzita wetlands in trays filled with peat moss in a shade house (20% shade) during the month of March (2009), when seedlings sprout in the field in our Mintzita springs model system. Once the plants reached between 5 and 20 cm tall they were transplanted individually to pots of 294 cm³ capacity containing peat moss. After two weeks, pots were randomly assigned to the ash addition treatments, and initial seedling size recorded for each plant. There were 19 treatment levels in this experiment, a control with no ash added and 18 ash addition treatments. The 18 treatment levels applied ash from either *Typha* or *Schoenoplectus* and from one of the 9 fertilizer combinations applied for growing the ash-producing plants. Each of the ash addition treatments had 19 replicates, each with 0.3 g of ash (the average amount of ash deposited after natural fires in our model system for an area equal to that of the pots, 36 cm²), for a total of 342 experimental units with ash added. The control had 38 replicates in a balanced orthogonal design, 19 for the *Typha* ash group and 19 to the *Schoenoplectus* ash group. The pots were watered with distilled water and harvested at 8 weeks. Final heights were measured and both aerial and root biomass was harvested, dried and weighed. If the plants developed a rhizome it was weighed separately. Rhizome starch content was analyzed colorimetrically (Hassid and Neufeld, 1964).

2.3. Phosphate and nitrate analysis

Soluble phosphate in the ash was assessed by adding 20 ml of distilled water to 0.2 g of homogenized ash and allowing it to soak for 24 h at 20 °C. After filtering the ash, phosphate was quantified by the ascorbic acid method (MAES, 1998) with a spectrophotometer (Genesis 6, Thermo Electron Corporation, Madison, Wisconsin) at 882 nm. An aliquot of each ash sample solution was analyzed for nitrates (Hach Nitrate Test Kit No. 1416100). Because nitrate concentrations were below or close to detection limits no further analyses for nitrogen were carried out.

2.4. Statistical analysis

Data were analyzed by ANCOVA and multiple regression techniques with Helmert contrasts (Crawley, 2007), using the initial plant size as a covariable to account for differences in size at the beginning of the experiment. Residuals were checked for compliance with the assumptions of the analyses. All analyses were carried out using R (R Development Core Team, 2009). All data are means and standard deviations unless otherwise noted. To test ash fertilization effects on *T. domingensis* seedling responses two
different approaches were taken. First, we assessed the effect of initial seedling size as a covariable and the source of the ash, *Schoenoplectus* or *Typha* leaves, regardless of the fertilization regime applied to the ash producing plants. The total number of experimental units (*Typha* seedlings) was 380, because there were 19 replicates for each ash group by species (342) and 38 controls (19 per ash group by species). The effect of the covariable was tested first in the model (1 degree of freedom) and secondly the effect of the origin of the ash, *Typha* or *Schoenoplectus* leaves (2 degrees of freedom), leaving 376 degrees of freedom. The degrees of freedom for rhizome variables differed because not all seedlings developed a rhizome. The second approach was to use as explanatory variables the initial seedling size and the concentration of P in the ash, for this analyses the multiple regression was implemented.

3. Results

3.1. Growth responses of fertilized plants for ash production

The two species responded similarly to the nutrient additions by increasing their above-ground biomass (Fig. 1). Only *S. americanus* had a perceptible above-ground response to P ($F_{(2,69)} = 3.7, P = 0.03$), because the treatments with P addition had 1.3 times more above-ground biomass than the treatment without this nutrient, but both showed a response to N. The increase for *S. americanus* with N addition was significant ($F_{(2,69)} = 90.4, P < 0.0001$), with the highest level having over 5 times the biomass of the control and 4 times the level of the lower level (Fig. 1). *Typha* had a similar pattern of N response ($F_{(2,69)} = 23.1, P < 0.0001$), but the magnitude of the differences were less and the pattern less clear because of the larger variation among the replicates.

The species produced different amounts of ash as expected from the differences in biomass (Table 1), although within species high amounts of aerial biomass did not necessarily correspond to high amounts of ash. Differences in soluble phosphate concentrations in the ash were detected through one way ANOVA between species ($F_{(1,16)} = 143, P = 0.002$), with the *S. americanus* ash containing almost twice as much soluble phosphate (0.68 ± 0.23 mg/g) as the *T. domingensis* ash (0.36 ± 0.08 mg/g). *S. americanus* ash showed more variability in phosphate concentration, ranging from 0.37 mg/g to 1.14 mg/g, whereas the *T. domingensis* ash (Table 1), had 0.27 mg/g to 0.48 mg/g. The overlap in phosphate concentration of the species occurred only between 0.37 mg/g and 0.48 mg/g. No relationships among the N:P fertilization treatments and the phosphorus concentration in the ash produced because of the great variability of the data.

3.2. Ash fertilization effects on *T. domingensis* seedlings

The experiment to test the effect of ash addition showed that *T. domingensis* seedlings responded to ash addition by growing taller and accumulating more biomass, both below and above ground, as the concentration of phosphate in the ash increased. Final height was significantly correlated with phosphate concentration in the ash ($R^2 = 0.44, P < 0.0001$, Fig. 2a). ANCOVA also indicates that the effect of the initial size of the plant is significant ($F_{(1,376)} = 21.11, P < 0.00001$). In agreement with our initial hypothesis the growth of *T. domingensis* differed with the species from which the ash was obtained ($F_{(2,376)} = 148.5, P < 0.00001$). *S. americanus* ash produced taller plants than both the control and *T. domingensis* ash, which did not differ. Leaf dry weight (Fig. 2b) and root dry weight (Fig. 2c) were also correlated with phosphate concentration in the ash, the former more strongly ($R^2 = 0.63, P < 0.0001$), than the latter ($R^2 = 0.58, P < 0.0001$), but both showed a better correlation than height growth. ANCOVA indicates that the effect of the initial plant size is significant for leaf dry weight ($F_{(1,376)} = 210.4, P < 0.00001$), and root dry weight ($F_{(1,376)} = 195.6, P < 0.00001$). After accounting for the effect of the initial plant size, the effect of the origin of the ash is still significant for leaf dry weight ($F_{(2,376)} = 106.0, P < 0.00001$). Plants that received *S. americanus* ash had greater leaf biomass than those of the control or of the *T. domingensis* ash treatment. The *S. americanus* treatment had the greatest mean root dry weight ($F_{(1,376)} = 56.1, P < 0.00001$), but with the *Typha* treatment mean root biomass being statistically indistinguishable from either the *S. americanus* or control treatment means (Fig. 2c). The root:shoot ratio decreased as the concentration of phosphate in the ash increased (Fig. 3). ($R^2 = 0.27, P < 0.0001$). ANCOVA indicates that the effect of the initial plant size is slightly significant for this variable ($F_{(1,376)} = 5.0, P = 0.026$), and the differences due to ash addition highly significant ($F_{(2,376)} = 63.1, P < 0.00001$), because plants from the control group differed from plant fertilized with *S. americanus* ash.

The number of plants that produced rhizomes differed among ash fertilization treatments. When no ash was added only 16 plants (42%) produced a rhizome, whereas 108 plants (63%) produced rhizomes when fertilized with *Typha* ash and 159 plants (93%) produced rhizomes when fertilized with *Schoenoplectus* ash (Chi
Fig. 3. Root-shoot ratio of *Typha domingensis* seedling fertilized with no ash added (C), ash from *T. domingensis* (T), and from *S. americanus* (S). In the left the relationship with phosphate concentrations in the ash, regardless of its origin is shown, to the right means and standard deviations of ash addition treatments are shown, different letters represent statistical differences (Tukey test).

Squared = 68, d.f. = 2, *P* < 0.00001). When comparing only plants that produced rhizomes, those fertilized with *Schoenoplectus* ash accumulated more rhizome biomass (0.07 ± 0.03 g), as reflected by dry weight measurements, than plants fertilized with *Typha* ash (0.04 ± 0.01 g) or plants with no ash added (0.03 ± 0.01), being the differences significant (*F* = 71.5, *P* < 0.00001). The ratio between rhizome biomass and total root biomass (that is roots plus rhizome), differed significantly among treatments. For plants with no ash added the ratio was 0.07 ± 0.02, for plants with the addition of *Typha* ash 0.01 ± 0.03, and for plants with *Schoenoplectus* ash 0.12 ± 0.08 (*F* = 9.5, *P* = 0.0001). Percent starch in rhizomes differed marginally among ash treatments (*F* = 3.61, *P* = 0.03), control plants had the lowest percentage 2.1 ± 0.7, followed by plants with *Typha* ash and *Schoenoplectus* ash (4.9 ± 4.3 and 4.9 ± 4.0, respectively).

4. Discussion

The results of this set of experiments are consistent with our hypothesis that fire can favor the spread of *T. domingensis* by facilitating seedling growth in burned areas dominated by *S. americanus*. The effect of ash addition on *T. domingensis* seedling growth was significant and proportional to the ash phosphate concentrations. Of course other factors are also important, most notably the removal of the plant canopy and litter that allow invasive seedlings to develop in a high light environment (Lindig-Cisneros and Zedler, 2002). Our results show that the nutrient effect may also be species-related in ways that facilitate the more invasive species. The growth of the *T. domingensis* plants was significantly higher where treated with *S. americanus* ash.

Some of the complexities in plant nutrient response to ash were revealed by comparing the results of the fertilizer experiment to the ash addition experiment. Overall, the phosphate ash concentration did not correspond to the amount of phosphate fertilizer added to the plants that produced the ash. For *T. domingensis* ash derived from plants growing under the different fertilizer regimes had very similar soluble phosphate concentrations. *S. americanus* ash had higher soluble phosphate concentration with higher levels of phosphate fertilizer only in the treatments with nitrate added. In the initial fertilizer experiment, both species had increased growth...
with increased N but little or no response to P fertilization. Yet in the ash addition experiment seedling growth did respond to P content of the ash.

We believe that these seemingly contradictory results follow from the discrepancies between artificial and more natural experimental conditions, and the fact that plants for the fertilizer experiment were produced from rhizomes and therefore began the experiment with some nutrient reserves probably tipped toward P since plants tend to conserve P more than N (Lambers et al., 1998). In contrast, in the ash experiment the seedlings had no rhizomes at the start and few of them developed them by the end of the experiment. Since the fertilization experiment was intended primarily to produce ash for the ash addition experiment, the lack of response to P is neutral with respect to the primary hypothesis under consideration, which was that T. domingensis seedling growth would differ with the source of the plant ash. Further separating the ash addition experiment from the fertilizer experiment is that the N added in the ash was probably minimal. It is known that burning causes large volatilization losses of N with the loss depending on the temperature. For T. domingensis, temperatures over 450 °C volatilize over 99% of this element (Quian et al., 2009a) whereas with temperatures up to 550 °C there is little volatilization of phosphorus from litter of several forest species (Gray and Dighton, 2006). Although fire temperature was not measured in our experiment, the fact that it was carried out outdoors under well ventilated conditions, as well as the results from the nitrate preliminary analyses suggest that temperatures were high and that most nitrogen was volatilized.

Differences in ash nutrient concentrations can be attributed to several factors including plant species, habitat nutrient availability, and live/dead ratio of aboveground biomass (Gray and Dighton, 2006). This study shows that for T. domingensis and S. americanus, within the range of fertilizer concentrations tested for growing the plants that produced the ash, the most important factor is the identity of the species. Although in our experiment practically all the aboveground biomass in all treatments of both species was already dead, the live/dead ratio of aboveground biomass was not manipulated and therefore this factor cannot be discarded as an important for determining final phosphate concentration in ash. The differences in phosphate concentration in the ash between the species most certainly can be attributed to their structural and chemical differences, as has been shown when comparing other plant species (Gray and Dighton, 2006). This suggests that in the field the timing of a fire with respect to the growth stage of the plants may be important in determining the nutrient content of the ash deposited.

The response of T. domingensis seedlings to ash fertilization is consistent with other studies showing that seedlings grown in soils in burned areas grow larger than seedlings grown in soils in unburned areas (Smith and Newman, 2001). After two months, seedlings fertilized with S. americanus ash in our experiment showed more aboveground biomass accumulation, bigger rhizomes and smaller root-shoot ratios than plants grown under control conditions, results similar to those found for two month old seedlings by Smith and Newman (2001), when testing soil from burned and unburned sites under natural conditions.

T. domingensis seedlings allocated less to roots when fertilized with more phosphorus-rich ash. Differential biomass allocation, to below and aboveground tissues, in response to nutrient availability is a well established fact for upland (Gusewell, 2004), and wetland plants (Gusewell, 2005). When nutrients are readily available lesser amounts of resources need to be allocated to below-ground tissues because they can be obtained from a small volume of soil. For T. domingensis, allocating more resources to above-ground tissues and accumulating more aerial biomass might provide several advantages, first it might allow young plants to show an escape strategy to inundation (Luo et al., 2011), that is rapidly grow leaves in response to increased water levels and second, it might provide a competitive advantage by preempting light at the expense of other slow growing species. In our experiment, plants with low root-shoot ratios produced larger rhizomes than plants with high ratios, the difference being a consequence of less root biomass in the former than in the later. For other wetland plants, allocation to storage tissues, such as rhizomes, allows for overwintering, fosters vegetative expansion, and for some species, such as Phalaris arundinacea, may confer a competitive advantage (Reinhardt Adams and Galatowitsch, 2005).

The implications of this experiment for explaining the observed trend of T. domingensis establishment in areas dominated by S. americanus in our reference system after fires are clear. At the Mintzita wetlands, the two dominant species responded differently to fire. The distribution of S. americanus was not affected by fire, but T. domingensis expanded its cover across the hydric gradient (Escutia-Lara et al., 2009a), by establishing large numbers of seedlings in areas dominated by S. americanus. It seems likely that part of the reason for this expansion is the rapid response of Typha to the fire-mediated release of P, increasing its growth considerably, thus possibly providing a competitive advantage. We continue to follow the long term population dynamics of both species but we believe that, the effect of nutrients, particularly phosphates liberated by fire has to be considered for management and restoration of wetlands where both species have historically coexisted.

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