Theor. Exp. Plant Physiol. (2016) 28:321–332
DOI 10.1007/s40626-015-0051-5

High aluminum availability may affect *Styrax camporum*, an Al non-accumulating species from the Brazilian savanna

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Received: 30 November 2015 / Accepted: 23 December 2015 / Published online: 25 January 2016

Abstract In the Cerrado vegetation, generally known as 'Brazilian savanna', aluminum (Al) accumulating and non-accumulating plants coexist, growing on soils that are acidic, poor in nutrients and rich in Al. Differing from Al-sensitive species, these plants are not expected to experience Al injuries. Using *Styrax camporum*, a non-accumulating plant, we recorded biometric variations in leaves, shoots and roots of young plants exposed to 0 and 1480 μM Al in a nutrient solution. Photosynthetic responses were measured bi-weekly over 91 days. Plants exposed to Al drastically reduced flushing, indicating that Al interferes with the functioning of the shoot apex. Aluminum caused low CO_2 assimilation rate, largely explained by low stomatal conductance, while Al-induced decrease in photochemical performance occurred only on some dates during the experiment. In addition, the absorbed Al was mostly retained in the roots. Although counter-intuitive, as this species grows on Al-rich soils, we noted that high Al availability impairs lateral root formation, causing an impact on water uptake and gas exchange rates of this species.

Keywords Al^{3+} · Cerrado woody species · Metal toxicity · Nutrient solution · Photosynthesis · Styracaceae

1 Introduction

The flora of the Cerrado is comprised of aluminum (Al) accumulating and non-accumulating species (Haridasan 1982; Souza et al. 2015a), which are distributed between savanna-type (cerrado *sensu stricto*) and forest (Cerradão) physiognomies of this vegetation (Ratter et al. 1997). These physiognomies are mostly comprised of shrubs and trees that grow on dystrophic and acidic (pH < 4.0) soils with exchangeable Al saturation (m %) between 60 and 90 % (Ratter et al. 1997; Habermann and Bressan 2011, Souza et al. 2015b). In addition, these soils are limited in nutrients, mainly to P, Zn, Cu, and Mn (Haridasan and Araujo 1988; Haridasan 2008; Pivello et al. 2010).

Some Al-accumulating plants may show above 15,000 mg Al kg^{-1} dry leaves and these species

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belong to a few families occurring in the Cerrado: Melastomataceae (*Miconia* spp), Rubiaceae (*Palicourea rigida*), Vochysiaceae (*Callisthene sp.*, *Qualea spp*, *Salvertia convallariodora* and *Vochysia sp*) and Loranthaceae (*Passovia ovata* and *Psittacanthus robustus*) (Haridasan 1982; Haridasan and Araujo 1988; Andrade et al. 2011; Scalon et al. 2013). Al-accumulating species may constitute 35 % of the species found in a Cerrado *sensu stricto* remnant (Haridasan 1982), and 18 % of the species found in a Cerradão fragment (Haridasan and Araujo 1988). The rest of the non-herbaceous Cerrado plant community may be considered non-accumulating species, which show between 100 and 600 mg Al kg^{-1} dry leaves (Haridasan 1982; Souza et al. 2015a).

On the other hand, most Al-sensitive species are herbaceous (crop) plants (Silva et al. 2012) or trees that are not able to secrete Al-organic acid complexes at the root tip (Brunner and Sperisen 2013). In these sensitive species, the most conspicuous symptom is the inhibition of root growth (Horst et al. 2010; Sun et al. 2010) because the Al binds itself to the rhizodermis, increasing its rigidity while reducing the ability of outer cells to elongate (Kopittke et al. 2008). These plants also show reduced gas exchange rates, such as CO_2 assimilation rate (*A*), which could be considered an indirect/long-distance effect caused by the fact that toxic Al binds itself to root cell walls and can be permanently stored in this organ (Vitorello et al. 2005; Rangel et al. 2009). Sensitive plants exposed to Al accumulate 70–80 % more Al in roots than in leaves and shoots (Jiang et al. 2009; Yang et al. 2011). Some studies attribute Al-induced decrease in *A* to photochemical apparatus injuries, as evidenced by low values of electron transport rate (ETR), effective quantum yield of photosystem II (ΦPSII) and photochemical quenching (qP) in plants exposed to more than 1000 μM Al (Chen et al. 2005; Jiang et al. 2008; 2009).

As far as we are aware, there are no studies of Al effects on plant growth or photosynthetic performance in either Al-accumulating or Al-non-accumulating species from the Cerrado. Aluminum has also been suggested to have some unknown positive roles in chloroplasts of Al-accumulating species, as it has been histochemically evidenced in these organelles of *Q. grandiflora* and *Callisthene major* (Vochysiaceae) (Andrade et al. 2011). Moreover, some Al-accumulating species do not grow well and show leaf chlorosis when cultivated in eutrophic soils with low m % (Haridasan 2008). In this way, although no physiological role has been suggested for Al in these plants, accumulating and non-accumulating species coexist in the Cerrado, growing on acidic soils with m % >70 %, with no apparent damage to their organs or metabolism (Andrade et al. 2011). Therefore, Al is not expected to cause disturbances in growth, physiological responses or morphological changes in these plants.

Styrax camporum Pohl. (Styracaceae) is considered a non-accumulating species (Haridasan 1982), although we have observed between 1000 and 1500 mg Al kg^{-1} dry leaves in field studies (data not shown). It is a tree (3–8 m in height), naturally occurring in Cerrado areas, and it exhibits a wide distribution between Cerrado physiognomies (Kissmann et al. 2012). It has been observed in remnants of Cerradão, cerrado *sensu stricto*, and other forest-influenced environments within Cerrado areas (Nakajima and Monteiro 1987). Its seeds are dispersed during the dry season (April-August) and are relatively easy to germinate (Kissmann and Habermann 2013), growing into five-leaf plants within approximately eight months.

In the present study, we predicted that *S. camporum* plants are not sensitive to high soluble Al concentration (>1000 μM) in nutrient solution. We recorded biometrical variations of leaves, shoots and roots of plants exposed to Al over 91 days and the Al concentration in these organs at the end of the study. During this period, we also measured photosynthetic parameters, such as gas exchange rates, as well as photochemical performances.

2 Materials and methods

2.1 Plant material and experimental conditions

Mature fruits of *Styrax camporum* Pohl were collected from adult trees growing in a Cerradão area (37 ha; 22°15′S and 47°00′W) in the municipality of Corumbataí, state of São Paulo, southeastern Brazil. Fifteen eight-month-old plants (26 ± 1 cm in height) were obtained from seeds that germinated in May 2013. The roots of these plants were rinsed under tap water to remove substrate debris composed of organic substrate (Tropstrato florestal®, São Paulo, Brazil), sand and

oxisol (1:1:1; v:v:v), on which these plants grew in 2 L (black) plastic bags, inside a greenhouse. The intact plants were transferred to opaque plastic boxes (50 cm in length × 30 cm in width × 15 cm in height; 20 L), containing nutrient solutions with 0 and 1480 μM Al.

As far as we can tell, studies of young non-accumulating plants from the Cerrado under contrasting Al concentrations are not available, and we have not previously tested other Al concentrations for *S. camporum*. Therefore, we chose 1480 μM Al (40 mg L^{-1}) because most studies testing high [Al] on Al-sensitive plants have used more than 1000 μM: 1480 μM (Konrad et al. 2005; *Coffea arabica*), 2000 μM (Chen et al. 2005; *Citrus reshni*), 1600 μM (Jiang et al. 2008; *C. grandis*), 1200 μM (Jiang et al. 2009; *C. grandis*) and 1850 μM (Silva et al. 2012; *Secale cereale*).

We used a nutrient solution (Furlani and Furlani 1988) with a chemical composition based on Clark's solution (Clark 1975) that has been used to study Al toxicity in Al-sensitive tree species (Santos et al. 2000). However, we diluted its macro- and micronutrient concentrations by seven in order to resemble the nutrient composition of Cerrado soils (Habermann and Bressan 2011; Souza et al. 2015b). For example, Kopittke et al. (2010) also observed that the soil solution from an Australian acidic oxisol exhibits nutrient concentrations that are approximately seven-fold lower than those in Hoagland & Arnon's nutrient solution. Although nutrient concentrations in solutions (mass per liquid volume) cannot be compared with nutrient *exchangeable contents* measured in soils (ionic charges per volume of a solid matrix), we observed that this final nutrient solution showed no precipitation and induced no nutrient deficiency in *S. camporum* plants. The pH of the aerated solution was maintained at 4.0 ± 0.1. Nominal 1480 μM Al supply resulted in 1100 ± 5.3 μM Al, and nutrient concentrations were as follows. Macronutrients (in mM): NO_3^- 0.137; NH_4^+ 0.058; P, 0.0019; K, 0.123; Ca, 0.204; Mg, 0.047; S, 0.031. Micronutrients (in μM): Cl, 30.58; Fe (EDTA), 3.32; B, 1.19; Mn, 0.41; Zn, 0.10; Cu, 0.04; Mo, 0.04. In addition, when we tested the chemical composition of this solution on Geochem-EZ software (Shaff et al. 2010) it resulted in more than 85 % free Al^{3+} available. Solution pH was monitored daily (corrected to 4.0, if necessary) and replaced every 10 days.

The boxes stood on benches inside a greenhouse with semi-controlled conditions. During the experiment, the photosynthetic photon flux density (PPFD) inside the greenhouse was 782.03 ± 157.73 μmol photons m^{-2} s^{-1}, with photoperiod of approximately 13 h, and air temperature, 29.5 ± 1.9. Expanded polystyrene (Isopor®) 50 × 30 cm plates (2-cm thick) with five holes (2.5 cm in diameter) were floated on the nutrient solution in the boxes, and the plants were fixed in these holes with polyurethane foam strips that were placed around the plant collar.

2.2 Experimental design

Five plants were grown in the box with the nutrient solution containing 1480 μM Al, and five plants were grown in the box with the solution containing 0 μM Al. In addition, leaves of five plants were counted and their shoot and root lengths (cm), leaf area (cm^2) and biomasses of leaves, stems and roots were determined at the beginning of the study. After 91 days, these biometric parameters were thoroughly measured for plants from both boxes, and the Al concentration was also measured in roots, shoots and leaves.

At 0 (on the day of planting), 14, 21, 28, 42, 49, 56, 62, 70, 86 and 91 days after planting (DAP), the gas exchange and chlorophyll fluorescence were measured on the plants' leaves.

2.3 Photosynthetic parameters

CO_2 assimilation (A) and transpiration (E) rates, stomatal conductance (g_s) and intercellular CO_2 (C_i) were measured with an open gas exchange system (LI-6400xt; LI-COR, Lincoln, NE, USA). Water use efficiency (WUE) was calculated as A/E, according to Habermann et al. 2003. CO_2 concentration entering the leaf cuvette was 390 μmol CO_2 mol^{-1} air, as provided by the 6400-01 CO_2 mixer (LI-COR). Measurements were performed between 9:00 and 11:00 am (Feistler and Habermann 2012) on cloudless sky days, under natural fluctuation of air temperature and vapor pressure deficit (VPD) inside the greenhouse. The VPD inside the leaf cuvette was 1.5 ± 0.2 kPa, which means that the relative humidity in the (reference) chamber oscillated around 65 %. Photosynthetic photon flux density (PPFD) was supplied by an artificial LED light (10 % blue and 90 % red) source (6400-40 LCF, LI-COR), which was set to provide 1200 μmol photons m^{-2} s^{-1}, as this value saturates A in *S. camporum* leaves (Habermann et al. 2011).

Chlorophyll *a* fluorescence was measured with a portable modulated fluorometer (6400-40 LCF; LI-COR), which was integrated into the LI-6400xt gas exchange system. For calculating maximum quantum yield of photosystem II (PSII) (F_v/F_m), leaves were dark-adapted for 30 min (Bolhàr-Nordenkampf and Öquist 1993) with aluminum foils, before measuring the fluorescence. The saturating light pulse was 7000 μmol photons m^{-2} s^{-1} during 0.7 s. F_m and F_v are maximum and variable fluorescence in dark-adapted leaves, respectively. The effective quantum yield of PSII (Φ_{PSII}), was calculated as $(F_m' - F_s)/F_m'$, where F_m' and F_s indicate the maximum and the steady state fluorescence in light-adapted leaves, respectively. Apparent electron transport rate (ETR = Φ_{PSII} PPFD 0.5 0.85) was calculated, using 0.5 as the fraction of excitation energy distributed to PSII, and 0.85 as the fractional light absorbance. The proportion of open PSII reactions centers (qP) was measured as $(F_m' - F_s)/(F_m' - F_o')$ (Bolhàr-Nordenkampf and Öquist, 1993). We also calculated the light fraction used for PSII in photochemistry [$P = ((F_m' - F_s)/F_m')$], heat dissipation in the antenna [$D = 1 - (F_v'/F_m')$] and heat dissipation in reaction centers [$E = (1 - qP)$ (F_v'/F_m')], which were in accordance to Demming-Adams (1996). For these calculations, F_v' is the variable fluorescence between the maximal (F_m') and minimal (F_o') fluorescence from light-adapted leaves.

2.4 Biometric parameters

Lengths of stems (from plant collar to the shoot apex) and roots (from plant collar to the root tip) were measured with a ruler (cm) and the number of leaves, counted.

The leaves, stems (plus petioles) and roots of the plants were separated. Leaf area (cm^2) was measured with an area meter (LI-3100C, LI-COR). Leaf, stem and root samples were oven-dried at 60 °C to constant mass, and biomass of these organs as well as the total biomass were measured using analytical scale.

2.5 Aluminum concentration in roots, shoots and leaves

At 91 DAP, after measuring the biomass of plant organs, the samples were oven dried at 60 °C for 72 h, ground and digested in a solution of sulfuric:nitric:percloric acids (1:10:2, v/v/v). After digestion, Al concentrations were determined by using an atomic absorption spectrophotometer (Sarruge and Haag 1974) and were expressed as mg Al per kg dry plant material.

2.6 Data analysis

The variation of A, g_s, E, C_i, A/E, F_v/F_m, ΦPSII, ETR, qP, P, D, and E between both treatments were analyzed using a T test at 5 % level at every evaluation date (0–91 DAP). We used the same T-test at 5 % level to check the variation of the number of leaves, leaf area, shoot and root lengths, and biomass of leaf, shoot, root and total biomass between both treatments at 0 and 91 DAP and, for these same biometrical traits, between 0 and 91 DAP for each treatment individually. For leaf, shoot, root and total Al concentrations measured at 91 DAP, the same T-test at 5 % level was used to check for differences between both treatments.

We used an allometric bivariate analysis (standard major axis regression—SMA) to test the correlation between $A \times g_s$ and $E \times g_s$ for both treatments and the variations in slope and intercept between the treatments. Data were $\log_{10}$ transformed (Warton et al. 2006, 2012). Statistical procedures were performed in R software (R Development Core Team 2012).

3 Results

Aluminum affected roots more than stems and leaves because, in plants exposed to Al, leaves were green, fully developed and had the same shape as those from plants not exposed to Al (Fig. 1a, b). Roots, however, were visually affected by Al, as there was a lack of lateral (or fine) roots in plants exposed to 1480 μM Al, which appeared to invest more in coarse roots (Fig. 1d) in relation to plants not exposed to Al (Fig. 1c).

Plants exposed to Al did not sprout normally, as their average number of leaves was 55 % lower than the control plants at 91 DAP, and plants exposed to Al did not increase the number of leaves from 0 to 91 DAP (Fig. 2a). Consequently, the leaf area of plants exposed to Al was the same between 0 and 91 DAP (Fig. 2b). In addition, the leaf biomass was similar between both treatments at 91 DAP, but while the control plants exhibited a four-fold increase in leaf

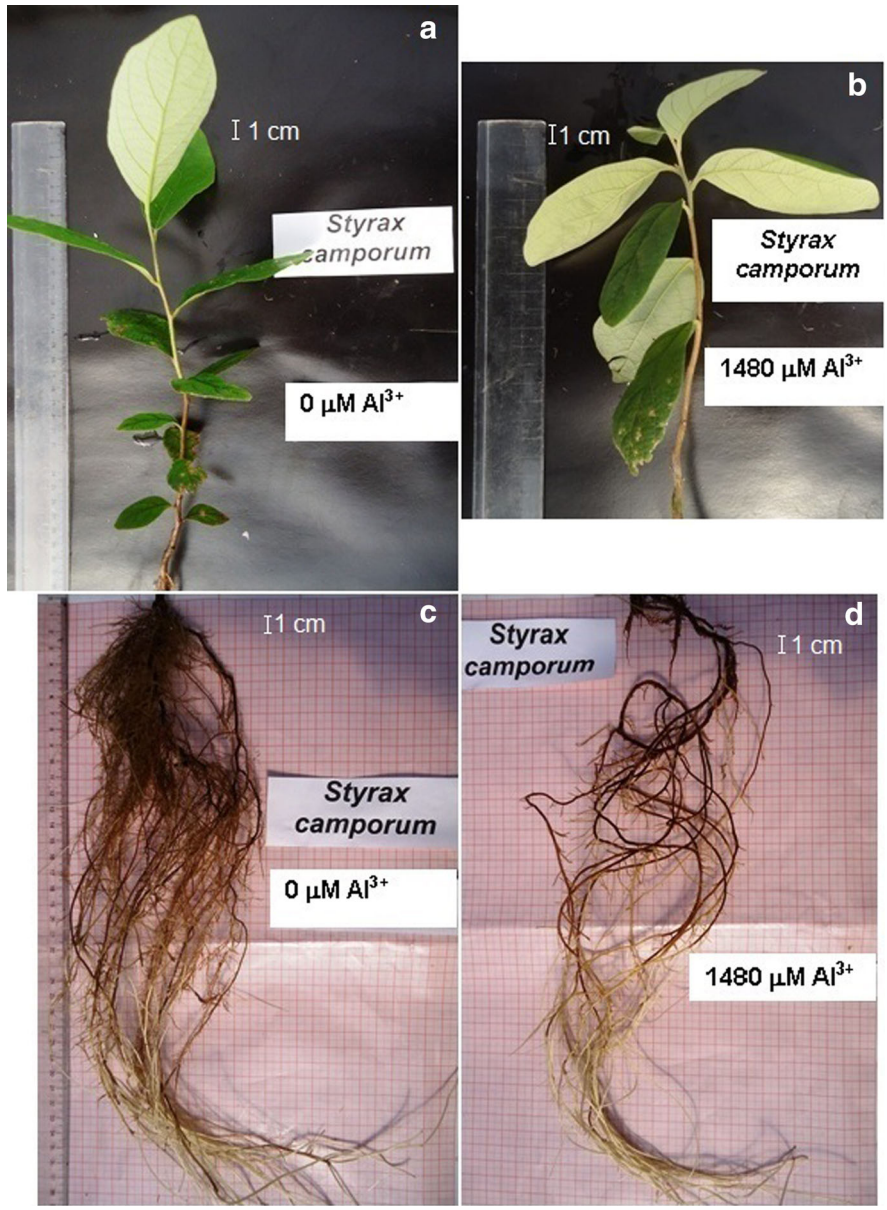

Fig. 1 Morphological details of shoots and leaves (**a**, **b**) and roots (**c**, **d**) of *S. camporum* plants grown for 91 days in nutrient solutions containing 0 (**a**, **c**) and 1480 (**b**, **d**) µM Al

biomass, plants exposed to Al showed an insignificant rise in this parameter between 0 and 91 DAP (Fig. 3a).

Aluminum also affected shoot growth because plants exposed to Al maintained the same shoot length after 91 days, while the shoot length of plants not exposed to Al increased 20 % during the same period (Fig. 2c). Although both treatments showed significant increases in their shoot biomasses from 0 to 91 DAP, plants not exposed to Al had a sharp five-fold increase in shoot biomass during the same period, but the shoot biomass, when comparing both treatments, was the same at 91 DAP (Fig. 3b).

The root length, when comparing both treatments, was the same at 91 DAP, but the roots of plants exposed to Al were 10 cm shorter than those from the control plants (Fig. 2d). Both treatments showed significant increase in their root biomasses from 0 to 91 DAP. In plants exposed to Al this parameter showed a ten-fold increase, while those not exposed to Al had a 16-times increase in root biomass during the same period (Fig. 3c).

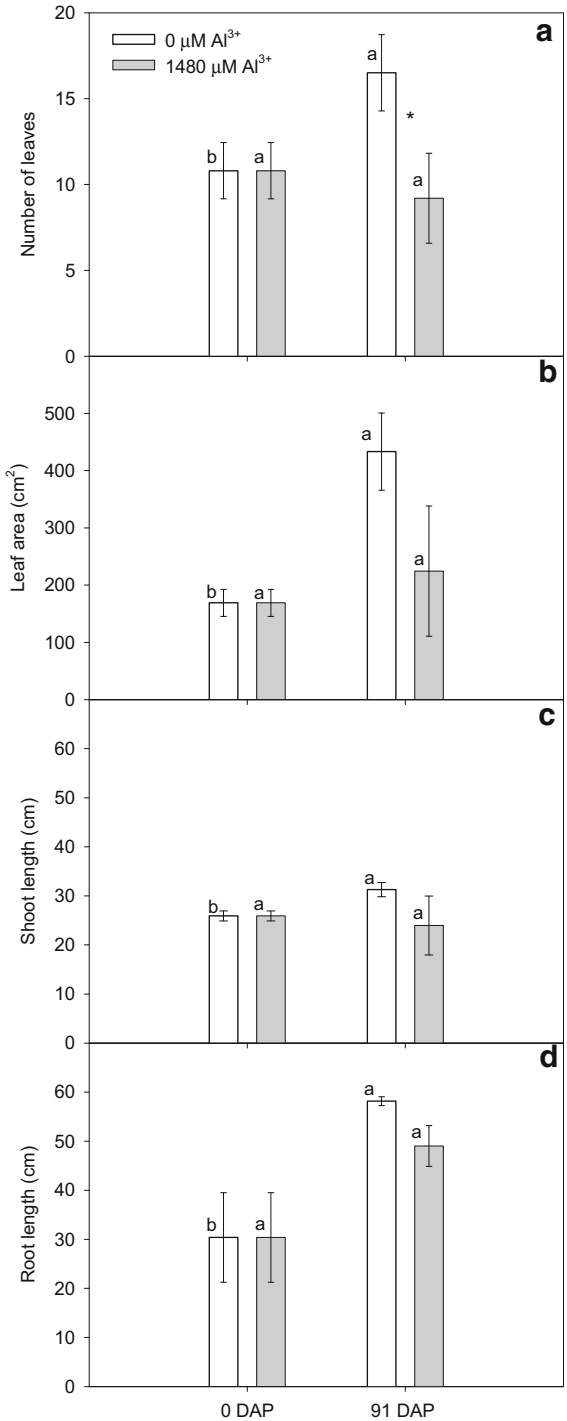

Fig. 2 Mean values (n = 5 plants) of biometric parameters (**a**–**d**) of *S. camporum* plants at 0 and 91 days after planting (DAP) in nutrient solutions containing 0 and 1480 µM Al. For the same treatment, *distinct letters* indicate significant differences ($P < 0.05$) between 0 and 91 DAP. *Asterisks* indicate significant difference ($P < 0.05$) between treatments at 91 DAP (*vertical bars* = s.d.)

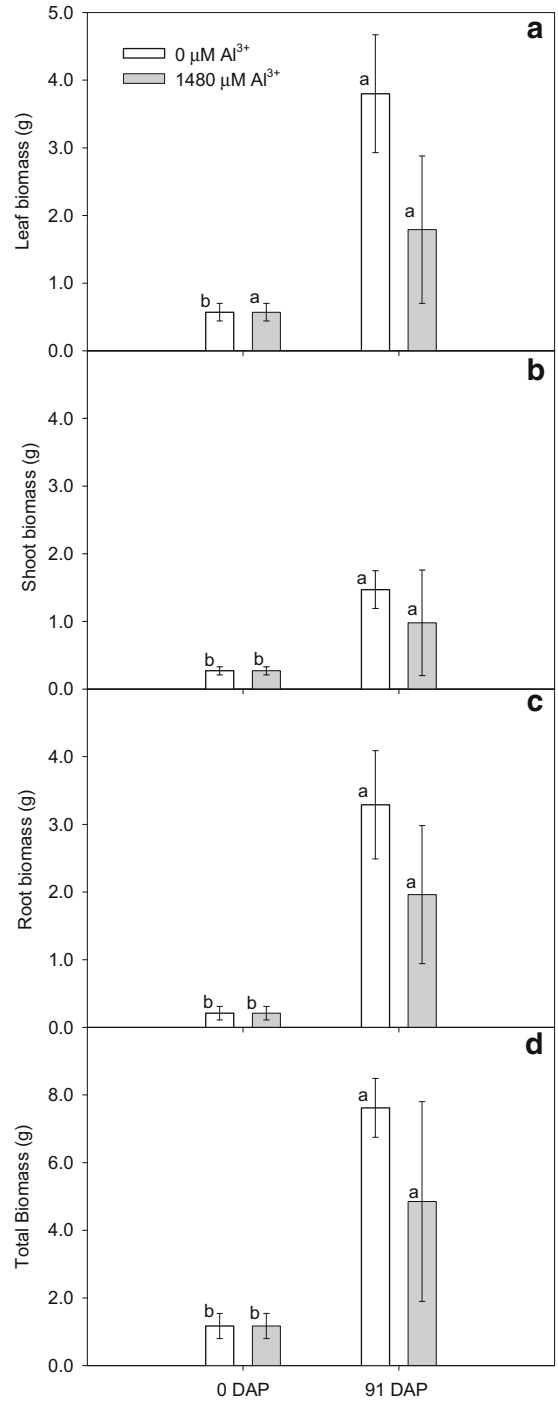

Fig. 3 Mean values (n = 5 plants) of biomass of organs (**a**–**c**) and total plant biomass (**d**) of *S. camporum* plants at 0 and 91 days after planting (DAP) in nutrient solutions containing 0 and 1480 µM Al. For the same treatment, *distinct letters* indicate significant differences ($P < 0.05$) between 0 and 91 DAP. Absence of *asterisks* indicates non-significant difference ($P > 0.05$) between treatments at 91 DAP (*vertical bars* = s.d.)

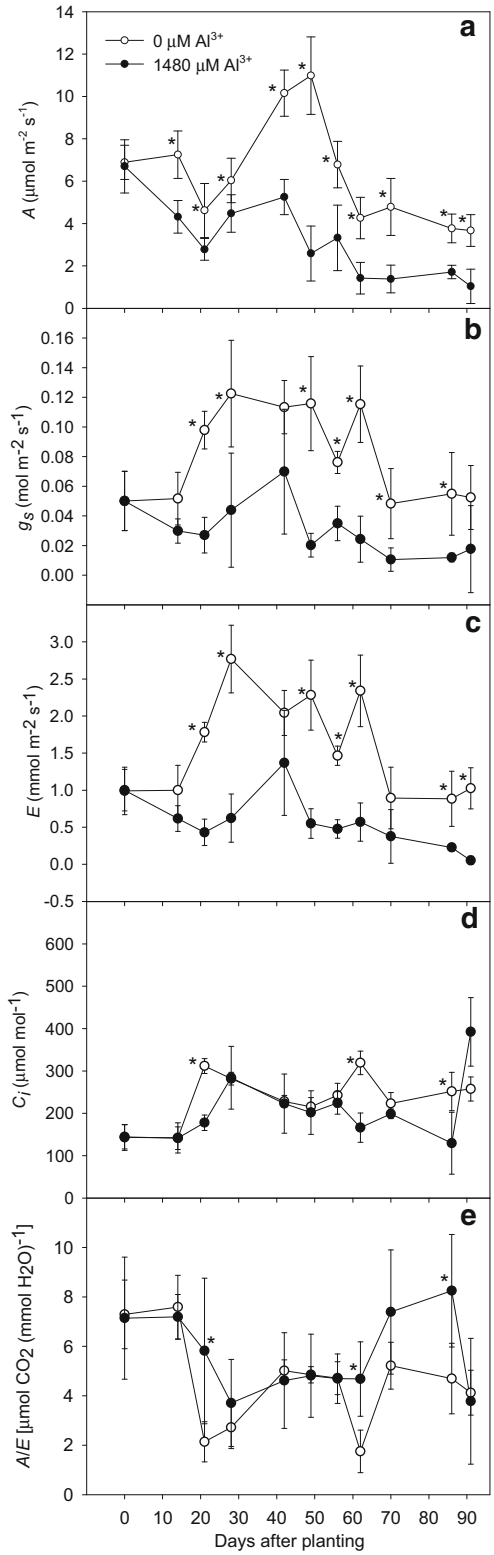

Fig. 4 Mean values (n = 5 plants) of gas exchange rates (**a**, **b**, **c**), intercellular CO_2 (**d**), and water use efficiency (**e**) of *S. camporum* plants grown for 91 days in nutrient solutions containing 0 and 1480 µM Al. *Asterisks* indicate significant difference ($P < 0.05$) between treatments at each evaluation date. (*vertical bars* = s.d.)

Aluminum reduced gas exchange rates. Despite the variability observed for these rates, *A* was lower in plants exposed to Al when compared to those not exposed to Al from 14 until 91 DAP (Fig. 4a). Values of g_s and *E* also reflected the response pattern observed for *A*. Except for the beginning and the end of the study, g_s and *E* remained lower in plants exposed to Al when compared to those not exposed to Al (Fig. 4b, c). Aluminum did not considerably affect the intercellular CO_2 (C_i), except at 21, 62 and 86 DAP when C_i was higher in plants not exposed to Al in relation to those cultivated with Al (Fig. 4d). Similarly, although not considerably affected by Al, the water use efficiency (*A*/*E*) was higher at 21, 62 and 86 DAP in plants exposed to Al when compared to those not exposed to Al (Fig. 4e). In addition, we observed significant correlations between $A \times g_s$ and $E \times g_s$ for both treatments (Fig. 5a, b). Both correlations differed for intercepts between treatments ($p < 0.05$).

Aluminum did not affect F_v/F_m (Fig. 6a). However, Al caused reductions in ΦPSII (Fig. 6b) and ETR (Fig. 6c), mainly between 14 and 56 DAP. Photochemical quenching was reduced in plants exposed to Al, but only at 14 and 21 DAP (Fig. 6d), and fractions of absorbed light used in photochemistry (P) were lower in plants exposed to Al at the beginning of the study and at 42 and 49 DAP (Fig. 7b). The heat dissipation in the antennas (D) was increased in plants exposed to Al at 21 (+3.5 %), 28 (+14 %), 49 (+27.5 %), 62 (+12 %), 86 (+13.8 %) and 91 (+13.7 %) DAP, while heat dissipation in the reaction centers (E) was higher in plants exposed to Al only at 21 DAP (+9.6 %) (Fig. 7).

At the end of the study, the plants exposed to Al showed higher Al concentration in relation to plants not exposed to Al, and this was a reflection of higher Al concentration found in all plant organs (Fig. 8). Interestingly, in plants exposed to Al, 69.5 ± 1.9 % of it was retained in the roots, while only 23.2 ± 3.4 % and 7.1 ± 1.9 % were retained in the shoots and leaves, respectively.

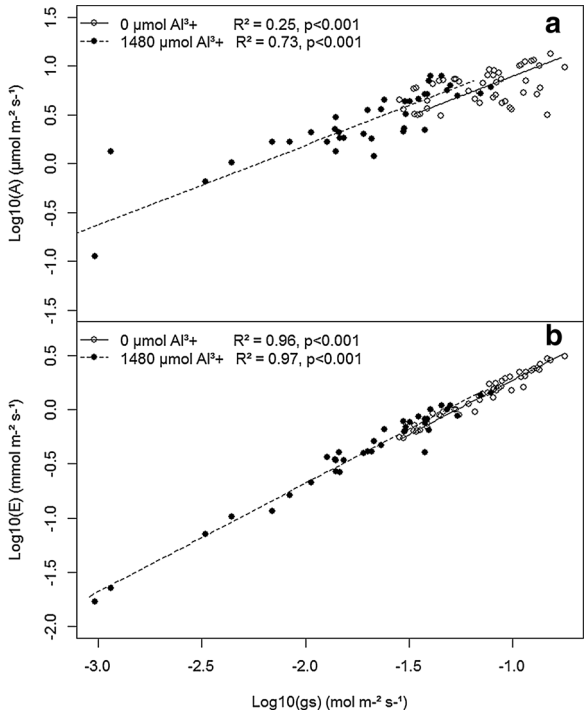

Fig. 5 Bivariate correlations between $A \times g_s$ (**a**) and $E \times g_s$ (**b**) for *S. camporum* plants grown for 91 days in nutrient solutions containing 0 and 1480 µM Al. Each *plot* represents reading performed on one plant measured throughout the experimental time

4 Discussion

Differing from our prediction, the results showed that high Al concentration in the nutrient solution affects the growth of *S. camporum*. Although the leaves of plants exposed to Al were fully expanded and had the same shape as those from plants not exposed to Al (suggesting no apparent toxicity to shoots), the number of leaves of plants exposed to Al remained the same between 0 and 91 DAP (Fig. 2a). Therefore, the smaller leaf number (Fig. 2a), leaf area (Fig. 2b) and leaf biomass (Fig. 3a) found in plants exposed to Al after 91 days, in relation to plants not exposed to Al are likely to be due to low leaf flushing. This indicates that Al may have interfered with the functioning of the shoot apical meristem. It is difficult to find studies of Al effects on leaves and shoots using simple data like number of leaves, mainly in native plants from environments where Al is not expected to be a toxic element, although Al saturation is extremely high in soils from these areas (Haridasan 2008). In *Cedrela*

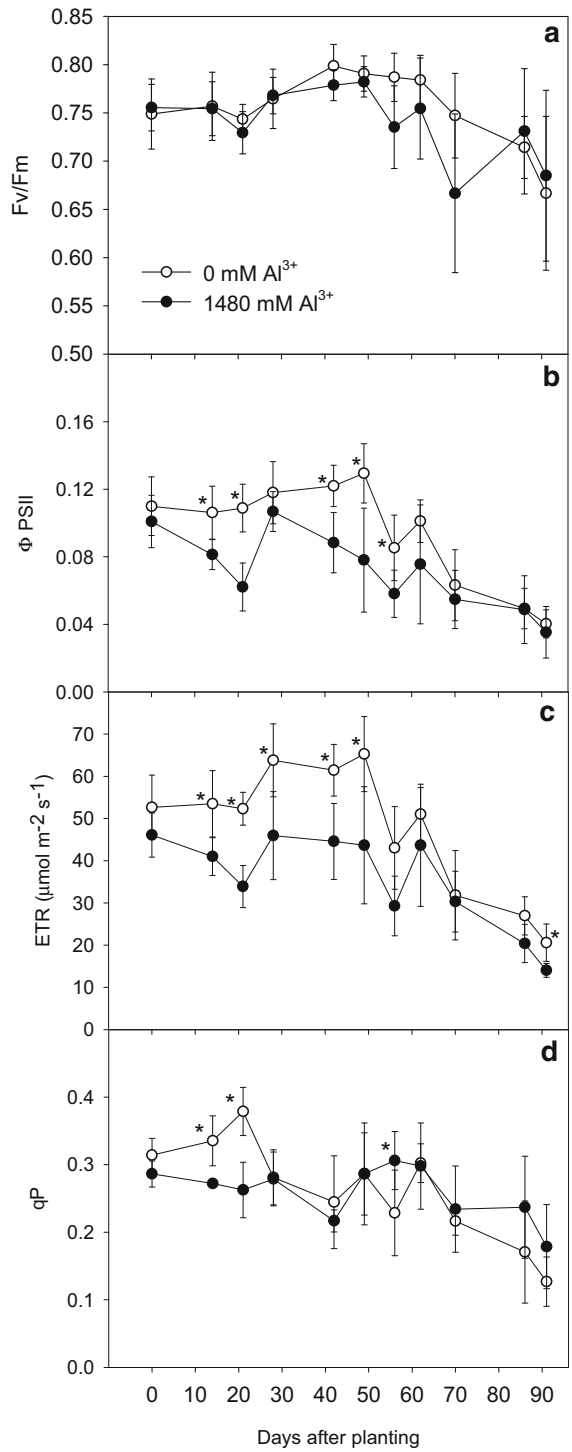

Fig. 6 Mean values (n = 5 plants) of chlorophyll a fluorescence parameters of *S. camporum* plants grown for 91 days in nutrient solutions containing 0 and 1480 µM Al. *Asterisks* indicate significant difference ($P < 0.05$) between treatments at each evaluation date. (*vertical bars* = s.d.)

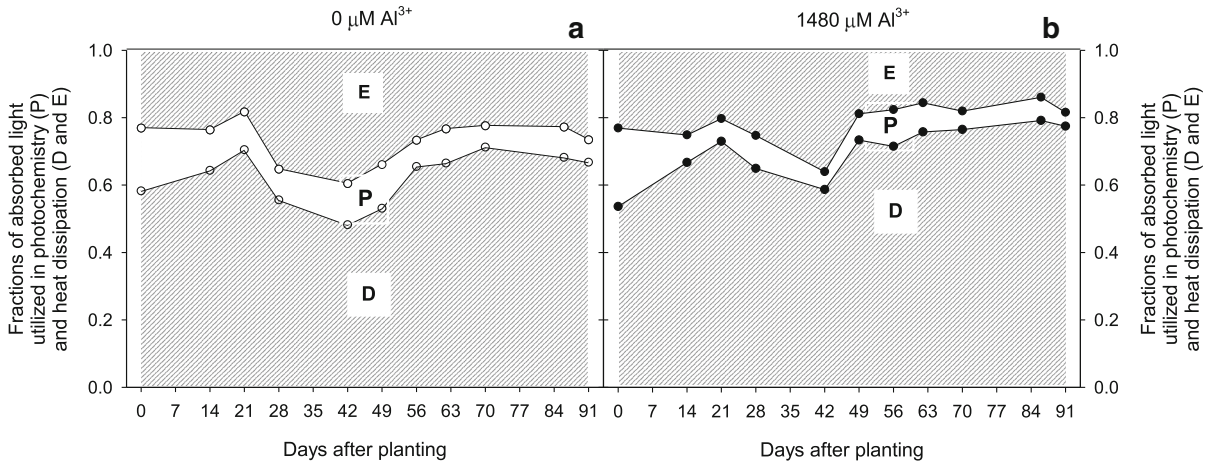

Fig. 7 Variations in fractions of absorbed light utilized in photochemistry (P), heat dissipation in the antenna (D) and in reaction centers (E) of PSII in *S. camporum* grown for 91 days under 0 (**a**) and 1480 μM Al (**b**). P values for each parameter between both treatments are presented below

Photochemical parameter	Days after planting										
	0	14	21	28	42	49	56	62	70	86	91
E	0.921	0.330	0.040	0.016	0.267	0.041	0.015	0.015	0.185	0.072	0.033
P	0.651	0.002	0.001	0.161	0.010	0.016	0.433	0.187	0.509	0.947	0.924
D	0.365	0.195	0.050	0.053	0.128	0.019	0.101	0.043	0.227	0.054	0.031

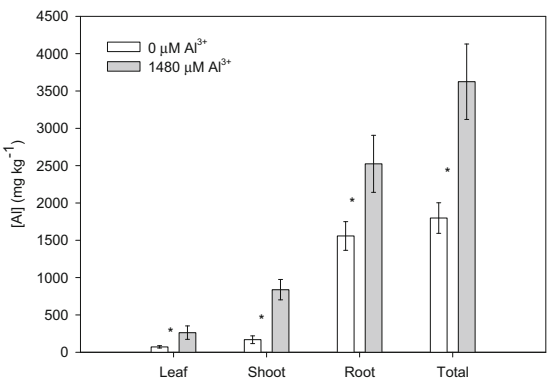

Fig. 8 Mean values (n = 5 plants) of Al concentration in leaves, shoots, roots, and in the whole plant of *S. camporum* grown for 91 days in nutrient solutions containing 0 and 1480 μM Al. *Asterisks* indicate significant difference ($P < 0.05$) between treatments at 91 DAP (*vertical bars* = s.d.)

odorata, *Heliocarpus americanus* and *Tabebuia chrysantha*, Al non-accumulating species from tropical forests in Ecuador, healthy leaf area decreased and leaf chlorosis increased with the increase of Al concentration (Rehmus et al. 2014). In rye, an Al-sensitive species, leaves were smaller after 3 weeks under 1110 μM Al (Silva et al. 2012). Therefore, *S. camporum* plants demonstrate some type of resistance to Al because after being exposed to high Al concentration for 91 days leaves were green and had the same size as those from plants not exposed to Al, despite the damage to their shoot apical meristem (low leaf flushing).

Aluminum is known to cause damage to apical meristems, interfering with cell division (Matsumoto 2000), but not to *shoot* apical meristems. The most important symptom of Al toxicity, in general, is the inhibition of *root* elongation (Horst et al. 2010; Sun et al. 2010). In Al-sensitive species, low root growth can be detected within hours under Al concentrations as low as 10 μM (Kopittke et al. 2008). In these plants, Al-induced decrease in root length may be of 60–80 % in relation to the root length of plants not exposed to Al (Blamey et al. 1987; Delhaize and Ryan 1995; Kopittke et al. 2008; Sun et al. 2010). In the present study, the roots of plants exposed to Al were only 10 cm shorter than those of plants not exposed to Al (Fig. 2d), representing a 15 % reduction in the root

length. Therefore, *S. camporum* exhibits some type of resistance to Al, as the typical Al-induced decrease in root growth exhibited by Al-sensitive species was not evidenced in this non-accumulating species from the Cerrado.

On the other hand, the most conspicuous symptom observed in the roots of *S. camporum* plants exposed to Al was the lack of lateral roots (Fig. 1d). Responsible for anchorage to the soil as well as for minerals and water supply (Kramer and Boyer 1995), lateral roots are *not* formed at the root meristem, but at the root maturation zone (Lavenus et al. 2013). Therefore, the root apex of *S. camporum* does not seem to be affected by Al, contrasting with the rapid and permanent damage to root meristems of Al-sensitive species (Kopittke et al. 2008). These results suggest that the root maturation zone is somehow affected by high concentration of Al in this species.

We also demonstrated that 69.5 % of the Al found in plants exposed to Al was retained in the roots. Aluminum was also found in plants grown in nutrient solution with no Al (Fig. 8), but this is a common observation in similar studies using (Al-sensitive) crop plants (Jiang et al. 2009; Yang et al. 2011). Root Al retention has already been reported for crop plants (Vitorello et al. 2005). In *Citrus grandis*, 70–80 % of the Al was found in roots (Jiang et al. 2009; Yang et al. 2011). *S. camporum* is a non-accumulating plant but, as we have already observed 1000–1500 mg Al kg^{-1} dry leaves in field studies, we expected to find Al in the leaves of plants of the present study. We did, but almost 70 % of the Al was retained in their roots and only 7.1 % in their leaves. In the field, *S. camporum* plants grow on soils showing m % between 60 and 90 % (Haridasan 2008; Andrade et al. 2011; Habermann and Bressan 2011). Although m % and Al concentration in nutrient solutions are not comparable, it is possible that (somehow) Cerrado soils are not toxic to non-accumulating plants when compared to nutrient solution containing Al, as demonstrated in the present study. In addition, no studies have demonstrated, so far, *whether* Al is retained (and in which proportion), or not, in the roots of *S. camporum* trees in the field. We did not anatomically/histochemically investigate possible sites of Al deposition for the Al we found retained in the roots of *S. camporum* plants but, apparently, the Al stunts lateral root induction in this species. This lack of lateral roots may explain the 60 % lower root biomass increment between 0 and 91 DAP in plants exposed to Al when compared to plants not exposed to Al (Fig. 3c). In addition, this lack of lateral roots may have interfered with the water uptake, which could be associated with the low gas exchange rates.

Therefore, our results also suggest that the reason behind the reduced gas exchange rates in *S. camporum* plants exposed to Al is diffusive, i.e. dependent on early stomatal closure (Chaves 1991; Chaves et al. 2002). Carbon assimilation was reduced in plants exposed to Al during most of the experiment (Fig. 4a), and the low g_s could explain their low *A* (Fig. 5a) and *E* (Fig. 5b) values. Thus, it seems that Al inhibits the formation of lateral roots at the maturation zone. Since lateral roots are responsible for water uptake (Kramer and Boyer 1995), the Al-induced decrease in lateral root formation might have caused a lack of water supply to the mesophyll, which eventually led to low g_s, and had an impact on gas exchange rates (Fig. 4a–c). Some studies (Chen et al. 2005; Jiang et al. 2008; Konrad et al. 2005; Silva et al. 2012) carried out on Al-sensitive species have reported 30–80 % decrease in g_s in plants exposed to Al. Samac and Tesfaye (2003) and Vitorello et al. (2005) defend that, in Al-sensitive plants, Al stunts the primary root and inhibits lateral root formation, which would lead to reduced water uptake. Therefore, it is possible that a similar sequence of responses (low *lateral* root formation → low water uptake → low g_s and gas exchange rates) might have occurred with *S. camporum*.

Low photochemical performances, such as reduced ETR, qP and ΦPSII have been observed in Al-sensitive plants when exposed to this metal, which could explain the low *A* observed in these crop plants (Chen et al. 2005; Konrad et al. 2005; Jiang et al. 2008; 2009). In the present study, low photochemical performance in plants exposed to Al included low ΦPSII and ETR (between 14 and 56 DAP), low qP (at 14 and 21 DAP), and increased D (between 21 and 91 DAP). However, (low) F_v/F_m, an indicator of damage to the photochemical apparatus (Baker 2008) was unchanged between the treatments (Fig. 4a). Healthy *S. camporum* plants under water deficit (leaf water potential = −3.2 MPa) also exhibit attenuation of photochemical performances (although F_v/F_m is stable at 0.78 ± 0.2), and under such conditions this species has to cope with relative excessive PPFD (Feistler and Habermann 2012). Moreover, while D

increased by 3–27.5 % in plants exposed to Al (Fig. 7b), g_s values decreased by 72 % (21 DAP), 64 % (28 DAP), 82.5 % (49 DAP), 54 % (56 DAP), 78.9 % (62 DAP), and 78 % (at 70 and 86 DAP) in these plants (Fig. 4b). The positive relationships between A x g_s (Fig. 5a) and $E \times g_s$ (Fig. 5b) for both treatments also reinforce that A was under diffusive (stomatal) control. Therefore, as observed for Al-sensitive species, in *S. camporum* plants the Al might have attenuated the photochemical performance, but the participation in such attenuation is not so important as the reduced g_s caused by Al in these plants. Consequently, it is more reasonable to assume that the low photochemical performance in *S. camporum* plants exposed to Al could be a mechanism to dissipate excessive energy due to a lack of water supply to the mesophyll, which led to a significant stomatal closure.

One may still argue that most photosynthetic parameters showed a reduction after 50 DAP, and that there was a considerable variation in gas exchange and photochemical parameters throughout the experiment (Figs. 4, 6). However, these variations were similar for both treatments. In addition, from 0 to 50 DAP, the mean air temperature was 30.2 ± 1.3 °C, dropping to 27.7 ± 1.5 °C after 50 days. Most importantly, A, g_s and E remained higher in plants not exposed to Al when compared to plants exposed to Al (Fig. 4a–c), reiterating that gas exchange rates seemed to be largely reduced by Al, even under variable conditions.

This may be the first report of Al effects on a non-accumulating species from the Cerrado, which would not be expected to experience injuries caused by Al. Therefore, we suggest further investigation with this species, such as Al dose–response experiments. Differing from our hypothesis, high soluble Al concentration in nutrient solutions seems to affect the growth of *S. camporum*. It interferes with the shoot apex, as plants exposed to Al drastically reduced flushing. However, in contrast with Al-sensitive species, Al is not a stressful factor to the root tip, but to the root maturation zone. It seems to disturb the formation of lateral roots and, consequently, water uptake is reduced, causing a lack of water supply to the mesophyll, which would explain the low g_s and reduced gas exchange rates.

Acknowledgments We acknowledge the São Paulo Research Foundation (Fapesp; Grant #2012/01351-9 to GH) and the Brazilian National Council for Scientific and Technological Development (CNPq; Grant #474169/2013-8 to GH) for the financial support. OFAA Banhos acknowledges the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a PhD scholarship. MC Souza acknowledges FAPESP for a Postdoc fellowship (Grant #2013/21293-6). G. Habermann acknowledges CNPq for a research productivity fellowship (Grant #308902/2014-9).

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