# Anatomic prerequisites for internal root aeration of three tree species of the Amazonian inundation forest

by

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(Accepted for publication: March, 2006).

## Abstract

Many tree species of the Amazonian inundation forest not only survive long periods of flooding, they are also able to continue shoot and root growth, flowering, and fruiting despite the unfavorable conditions of the oxygen-depleted soil. Internal root aeration is an effective adaptation mechanism that maintains the high-energy status required by root cells - to support growth and development. In the present study, internal root aeration was investigated in three Amazonian species by using oxygen microelectrodes to measure oxygen concentrations in the root cortex and rhizosphere, and light and fluorescence microscopy to analyze lacunar shape, extent, and distribution, as well as suberin incrustations in the exodermis. A highly porous gas transport system from the shoot to the roots is the prerequisite for an effective internal oxygenation. The results showed that the roots of *Nectandra amazonum* were not oxygenated, although large, regularly distributed intercellular spaces were observed in the root cortex. The roots of *Triplaris pyramidalis* were moderately supplied with oxygen via irregularly distributed, developing cavities in the cortex. Internal oxygen transport within regularly distributed lacunae (aerenchyma) in the root cortex of *Pseudobombax munguba* was reflected by the high concentration of oxygen measured in this species. In the roots of *Triplaris pyramidalis*, internal oxygen was conserved due to the formation of a hypodermal suberin barrier. By contrast, in *Pseudobombax munguba*, the absence of a suberized layer resulted in the diffusion of oxygen into the rhizosphere. The tree species display three different strategies, although they grow in the same habitat: no internal aeration at all, internal aeration without loss of oxygen to the rhizosphere, and internal aeration with massive loss of oxygen to the rhizosphere.
Keywords: **Anoxia, aerenchyma, flood adaptation, radial oxygen loss, root aeration.**

## Resumo

Muitas espécies arbóreas das florestas alagáveis da Amazônia não somente conseguem sobreviver longos períodos de inundação, as espécies também são capazes de manter o crescimento de brotos e raízes, floração e frutificação apesar das condições desfavoráveis de um solo esvaziado por oxigênio. Aeração das raízes internas é um mecanismo eficaz de adaptação para manter o estado de energia necessitada pelas células da raiz - para suportar o crescimento e desenvolvimento. No estudo presente, aeração das raízes internas foi analisada para três espécies amazônicas usando microelectrodos de oxigênio para medir a concentração de oxigênio no córtex da raiz e na rizosfera, e microscopia de luz e florescência para analisar a forma do aerênquima, extensão, e distribuição, também como incorporação de suberina na exodermis. Um sistema com muitos poros é pré-requisito para o transporte de gás dos brotos para as raízes para uma

oxigenação interna com eficiência. Os resultados indicam que as raízes da *Nectandra amazonum* não foram oxigenadas, mesmo largas, espaços intercelulares com uma distribuição regular foram observados no córtex da raiz. As raízes da *Triplaris pyramidalis* foram moderadamente suportadas com oxigênio através de cavidades com uma distribuição irregular no córtex. O transporte interno de oxigênio dentro de um aerênquima regularmente distribuído no cortes da raiz do *Pseudobombax munguba* foi refletido pela concentração mais alta de oxigênio medido nesta espécie. Nas raízes da *Triplaris pyramidalis*, oxigênio interno foi conservado através da formação de uma barreira hypodermal de suberina. Em contraste, no *Pseudobombax munguba*, a ausência de um estrato com suberina resultou na difusão de oxigênio para a rizosfera. As três espécies representam três estratégias diferentes, mesmo que elas crescem no mesmo habitat: nenhuma aeração interna, aeração interna sem perda de oxigênio para a rizosfera, e aeração interna com perda massiva de oxigênio para a rizosfera.

## Introduction

Morphologic, anatomic, metabolic, and functional adaptations of plants to permanent or periodic hypoxic or anoxic growth conditions as a result of flooding have been studied intensively, and the results reviewed in several publications (ARMSTRONG 1979; CRAWFORD & BRAENDLE 1996; JACKSON & COLMER 2005; KOZLOWSKI 1997; LÖSCH & BUSCH 2000; PAROLIN et al. 2004; PEZESHKI 2001; VISSER et al. 2003). Most of those studies focused on the plants' above-ground organs, since although plant roots are strongly affected by anoxic soil conditions, they are more difficult to examine. Studies of the specific adaptations of tree roots are even less numerous than those evaluating aquatic and graminaceous species. Intensively studied woody species include *Alnus* species, *Salix* species, *Fraxinus excelsior*, other tree species in temperate regions (ARMSTRONG 1968; DITTERT et al. 2006; EWING 1996; HARRINGTON 1987; IREMONGER & KELLY 1988; JACKSON & ATT-WOOD 1996; KOZLOWSKI 1997; SIEBEL et al. 1998), mangroves at different sites (ALLAWAY et al. 2001), and a few tropical tree species (DE SIMONE et al. 2002, 2003; JOLY 1996; LOBO & JOLY 1998; LOPEZ & KURSAR 1999; PAROLIN 2001; PAROLIN et al. 2002; WALDHOFF et al. 1998). The results of those investigations revealed species-specific differences in adaptations to waterlogging and flooding. Some trees avoid oxygen deficits in their roots by internal aeration, which is an important survival strategy during unfavorable growth conditions. In contrast to anaerobic metabolism, root aeration, as a prerequisite for root respiration and radial loss of internally transported oxygen, is of particular importance for energy supply, nutrition uptake, and protection against reduced phytotoxic ions, and thus for plant survival (JANIESCH 1991). The production of new roots, leaves, flowers, and fruits by numerous species of the Amazonian inundation forest during the aquatic phase (SCHÖNGART et al. 2002) points to their optimal energy supply and suggests internal aeration processes.

Internal oxygen transport has been detected through measurements of the redox potential of the rhizosphere (DITTERT et al. 2006), the reduced-dye technique (ARM-STRONG & ARMSTRONG 1988), and cylindrical polarographic electrodes (ARM-STRONG 1994), but these methods require the loss of oxygen into the rhizosphere. Oxygenation in the root tissue has been shown by means of oxygen microelectrode measurements (ARMSTRONG et al. 2000). DE SIMONE et al. (2002) investigated root aeration in three tree species of the Amazonian inundation forest by this *in vivo* technique. Differences in aeration were shown in a comparison of the species, *Salix martiana, Tabernaemontana juruana*, and *Laetia corymbulosa*, growing in the same environment (DE SIMONE et al. 2002, 2003). The aims of the present study were: (1) to broaden the

set of described Amazonian tree species, (2) to confirm known aeration strategies, and (3) to demonstrate further differences in aeration mechanisms. Therefore, the root morphology and anatomy of three other common Amazonian tree species growing in the same habitat were examined under simulated flooding conditions. Particular attention was given to the capacity for internal root oxygenation, the nature of the oxygenated roots, and the occurrence of radial oxygen loss to the oxygen-depleted rhizosphere.

## Material and methods
### Plant material
Experiments were carried out with young tree cuttings: the brevi-deciduous species *Triplaris pyramidalis* JACQ. (Polygonaceae), the evergreen species *Nectandra amazonum* NEES (LAURACEAE), and the stem succulent *Pseudobombax munguba* (MART. & ZUCC.) DUGAND (Bombaceae). *T. pyramidalis* mainly grows in the high várzea (Amazonian white-water forest) although individual trees are established at lower flood levels. The trees are inundated at least 50-100 days per year. *N. amazonum* grows in the low várzea, with an inundation period of at least 100-150 days per year. *P. munguba* is distributed along the entire flood-level gradient, but most of the trees are located in the low várzea (WITTMANN et al. 2004). All species are subject to long-term flooding in their natural habitats.

Cuttings were derived from 2-year-old trees grown from seeds collected in Central Amazonia in a climate-controlled greenhouse. The cuttings were rooted in standard potting soil and transferred after 8-12 weeks to a climate chamber. The plants grew under stable climate conditions resembling those of Central Amazonia: 70-80 % relative humidity, day/night regime of 12/12 h (PAR 50 $\mu$mol m$^{-2}$ s$^{-1}$), 32/28 °C day/night temperature. In addition, the cuttings were illuminated with 100 $\mu$mol m$^{-2}$ s$^{-1}$ by four high-pressure mercury lamps (HQL-R, 80 W, OSRAM, Germany) for the middle 8 h of the day.

To simulate stagnant water and anoxic growth conditions in the rhizosphere, the roots of the cuttings were washed carefully under lukewarm tap water and planted into agar medium in a glass basin. The solid agar (0.5 % w/v) was dissolved in a nutrient solution containing (mmol l$^{-1}$): NH$_4$NO$_3$ (3.0), MgSO$_4$ (0.5), CaCl$_2$ (1.5), K$_2$SO$_4$ (1.5), NaH$_2$PO$_4$ (1.5), and the following trace elements ($\mu$mol l$^{-1}$): H$_3$BO$_3$ (25), MnSO$_4$ (1), ZnSO$_4$ (0.5), (NH$_4$)$_6$Mo$_7$O$_{24}$ (0.05), Cu SO$_4$ (0.3), and FeEDTA (40). The pH was between 5.5 and 6. The cuttings were transferred to fresh medium at least once a week. The plants grew in the agar medium for at least 10 weeks and oxygen concentrations in the cortex of several roots were repeatedly measured (Tab. 1). The glass basin was completely wrapped in black paper to prevent illumination of the roots and agar medium.

### Oxygen measurements
Oxygen was measured with highly sensitive and specific microelectrodes (Ox 25, tip diameter 25 $\mu$m, Unisense, Aarhus, Denmark). Details on the operating mode of these miniaturized Clark-type electrodes (REVSBECH 1989) and on the measuring technique were provided in DE SIMONE et al. (2002).

For oxygen measurements, the cuttings were planted into agar medium consisting of a solid layer (2 %, w/v) as a lay-on for the roots and an upper layer (0.5 %, w/v) surrounding the roots. The latter allowed the simulation of water-filled soil pores and the establishment of oxygen profiles. Selected roots were positioned 0.5 cm from the wall of the glass basin (250-500 ml depending on the root ball) to optimize observation by means of a magnifying glass. A water layer of 1-2 cm covered the agar surface to prevent shrinkage of the agar and to simulate flooding conditions. The microelectrode was gradually driven by means of a mechanical micromanipulator through an agar layer of 2.5-3.5 cm before it touched the root surface. Oxygen profiles were recorded 20 h after the cutting had been inserted in fresh agar. Even though the microfloral content of a stagnant solution is less than in most soils (WIENGWEERA et al. 1997), at the time of measurement oxygen was completely consumed in the basin due to root respiration and developing microbial activity beneath the top centimeter of agar. Both the respiration rate of the roots and the growth rate of microorganisms introduced into the agar by the roots were high because of the high temperature (28-32 °C) in the climate chamber. Oxygen profiles obtained from around the root ball verified

the anoxic conditions; consequently, oxygen liberated near the roots was exclusively derived from root oxygen. The microelectrode was inserted into the root tissue (100-200 μm) until a constant cortical oxygen value was obtained. To the extent possible, the electrode was positioned at right angles to the root surface. Radial profiles in the rhizosphere and concentrations in the cortex were recorded leastwise at two measuring points, i.e., 1 cm behind the root tip and 2-3 cm from the root origin.

## Microscopy

The roots in which oxygen had been assayed using microelectrodes were subsequently harvested for microscopy. The lacunae in the root cortex were visualized by light-field microscopy. Presumed suberin incrustations, which may act as a selective barrier for gas transport in the epidermal and subepidermal cell walls, were observed by fluorescence microscopy (Leitz, Laborlux, Germany). Photos were taken with a digital camera (DFC 320, Leica, Germany).

## Light microscopy

Tissues of the root segments were fixed in 4 % paraformaldehyde in 100 mM phosphate-buffered saline (PBS), pH 7.0, for at least 24 h. The root segments were washed three times with PBS (100 mM) and transferred into liquid agar (2 % w/v), which was then allowed to solidify. The resulting agar cuboids containing the roots were cut and the segments placed in Histoform S embedding mold (Kulzer, Germany) for fixation as follows: The samples were dehydrated in a graded ethanol series of 20, 40, 60, 80, and 100 % (30-min/step), pre-infiltrated in an ethanol/glycolmethacrylate mixture (1:1) for 24 h, and embedded in pure glycolmethacrylate (Technovit 7100, Kulzer, Germany), as described by RUETZE & SCHMITT (1986). The carrier plates were then glued to the histology blocks (Technovit 3040, Kulzer, Germany). Sections of 3-4 μm thickness were cut with a rotation microtome (RM 2165, Leica, Germany) with disposable 45°-knives (Histoknife, Kulzer, Germany), floated on a water surface at room temperature, and transferred to microscope slides. The polymerized sections stuck to the glass surface after drying on a warming tray (50 °C). Root cross-sections were stained in 0.05 % toluidine blue O (w/w) for at least 1 min. Glycerin-gelatin was used to seal the cover slips. Cross-sections were viewed by light-field microscopy.

## Fluorescence microscopy

Transverse free-hand sections, cut using a razor blade (Wilkinson, Sword Classic, Germany), were stained for suberin and then incubated in 0.05 % toluidine blue O (w/w) in 100 mM PBS, pH 6.0, to quench lignin and suberin autofluorescence. The sections were stained with 0.1 % neutral red (w/w) in 100 mM PBS, pH 6.0, for 1 min and washed with tap water. This procedure yielded suberin-specific fluorescent probes (LULAI & MORGAN 1992). The cross-sections were transferred to a drop of tap water on microscope slides, and cover slips were placed over the samples. Blue-violet excitation (Leitz I 2/3, Germany; excitation filter 450-490, dichromatic beam splitter 510, and barrier filter 515) was used in all investigations of suberin deposition.

## Results

### Triplaris pyramidalis

After immersion of the young roots in agar, the young trees did not show symptoms of impaired growth. Roots that had been developed during aerobic conditions grew slowly under anoxic conditions until elongation ceased, at a length of about 6 cm. The formation of single lateral roots and of new roots under the latter conditions was observed. Between 0.8 and 2.5 mg oxygen $l^{-1}$ (mean value: 1.5 mg $l^{-1}$, Tab. 1) was measured in the cortex of all roots originating from the portion of the stem at least 4-5 cm below the water and agar layers (measuring point: 2-3 cm from the root base, length of the roots: 4-6 cm). Oxygen was detected at the outer root surface only along the 0.5-0.8 cm of the white apex and did not exceed 0.4 mg $l^{-1}$ (Fig. 1a). This slight radial diffusion of

oxygen occurring around the root apex was most likely not enough to create a detectable oxygen profile in the rhizosphere, which was populated with microorganisms. Oxygen profiles in the remaining, brownish part of the root (2-3 cm from the root origin) showed a very steep slope at the site of penetration of the root exodermis, suggesting that oxygen transport from the oxygenated root cortex to the rhizosphere was greatly restricted (Fig. 1a).

The anatomy of the cortex of *T. pyramidalis* roots was characterized by a large number of intercellular spaces, degeneration of the parenchymatic cortical cell walls, and numerous irregularly arranged, developing air lacunae. The latter are a prerequisite for oxygen transport (Fig. 2a).

Figure 2b shows the strongly fluorescent suberin deposits found in the radial and inner tangential cell walls of the hypodermis. In the apical region, there was a slight diffusion of radial oxygen ($\leq 1$ cm beyond the tip) but no suberin incrustations were seen (photo not shown). Suberin staining did not differ between deposits in the aerobically grown parts of the root (brownish part) and those in the anoxically grown fresh regions (whitish part).

## Pseudobombax munguba

Immersion of the young roots in agar not only allowed the growth of aerobically grown roots to continue, it also promoted the development of new, adventitious roots. Under the experimental conditions, roots of *P. munguba* reached a length of 10-12 cm. Oxygen microelectrode measurements in the proximity of these vital original roots and of the new roots demonstrated oxygenated layers along their entire lengths. Oxygen concentrations in the root cortex were around 3.5 mg l$^{-1}$ (measuring point 2-3 cm from the root basis, length of the roots: 5-12 cm; Tab. 1). The oxygen gradient between the cortex and the outer surface was very much less step, both in the white apex region and in the brownish part near the root origin, indicating massive radial oxygen diffusion from the well-oxygenated cortex to the rhizosphere (Fig. 1b).

Light microscopy examinations of the cortical tissue of *P. munguba* showed regularly arranged air lacunae (aerenchyma) of different shapes (Fig. 2c). In the aerenchyma, numerous ligaments remained. The most expanded lacuna, seen in cross-section, occupied nearly a quarter of the cortex (photo not shown).

Suberin deposits were not found at any site along the entire root in the rhizodermis, as determined by fluorescence microscopy (Fig. 2d). The lack of suberin incrustations corresponded well to the pronounced oxygen profiles in the agar surrounding the roots.

## Nectandra amazonum

Aerobically grown roots of *N. amazonum* did not elongate under anoxic conditions in oxygen-free agar medium and there was no development of new roots. Oxygen was not detected either outside or inside the roots (Tab. 1, Fig. 1c).

Anatomic examination of the cortical root tissue of *N. amazonum* showed regularly distributed, large, intercellular spaces but no extended lacunae (Fig. 2e).

Fluorescence microscopy demonstrated moderate suberin deposits in the cell walls of the hypodermis of *N. amazonum* (Fig. 2f). A weak fluorescence signal was visible in the radial and inner tangential cell walls. Portions of the hypodermis contained fewer suberin incrustations, suggesting pore formation in the hypodermal layer.

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## Discussion

In wetlands, soil flooding restricts soil-atmospheric gas exchange leading to rapid depletion of soil oxygen by roots, microorganisms, and soil reductants (PEZESHKI 2001). External supply of root cells with oxygen stops. Generally, internal oxygen transport is an important trait for both herbaceous (ARMSTRONG et al. 1994; COLMER 2003; COLMER et al. 2006) and woody (ANDERSEN & KRISTENSEN 1988; ARMSTRONG & ARMSTRONG 2005; DITTERT et al. 2006; JACKSON & ATTWOOD 1996, LI et al. 2006) plants, in that it compensates for local anoxic conditions. Root respiration that is restored by internal oxygen supply maintains the plant's high-energy status. It is thus essential for root growth, nutrient and water uptake, and the synthesis of structural substances as well as various secondary metabolites. A fraction of the root oxygen derived from internal transport can be lost by radial oxygen diffusion to the rhizosphere. This oxygen is of great importance for the well-being of the plant; for instance, it is used in the oxidative detoxification of reduced ions or gases in the rhizosphere (ARMSTRONG & ARMSTRONG 2001; JACKSON & ARMSTRONG 1999). Concurrently, the loss of oxygen into the anoxic surroundings means that there is less available for root metabolism. According to several authors, the restriction or control of radial oxygen loss by the formation of an anatomic barrier in the root exodermis along most of the root system is the predominant adaptive process (COLMER et al. 1998; KONČALOVA 1990; McDONALD et al. 2002).

The results of this study showed that different types of cortical root tissues carry out internal gas transport for root oxygenation via an interconnected lacunae system that extends from the aboveground organs to the roots. Microscopy of the root cortex revealed the common intercellular spaces in all species and the air lacunae. The latter varied in shape as well as extent in *T. pyramidalis* and *P. munguba*. Previous studies found that an increase in root porosity due to the development of aerenchyma leads to a reduction in both resistance to diffusive and pressurized gas transport and the number of cells, resulting in a decrease in metabolic oxygen consumption (JACKSON & ARMSTRONG 1999; COLMER et al. 2006; LI et al. 2006; WALDHOFF et al. 1998). Together, these processes imply that cortex oxygen concentrations increase with increasing root porosity. This was verified by measuring cortex oxygen concentrations with microelectrodes. High oxygen concentrations were found in the cortex of *P. munguba*. In this species, the root cortex contained many regularly distributed lacunae that appeared to be fully developed aerenchyma of lysigenous origin. By contrast, lower oxygen concentrations were found in *T. pyramidalis* roots. The irregularly arranged developing lacunae of this species lacked a complete aerenchyma; instead, single enlarged cavities and widened intercellular spaces were present. Oxygen was not detected in the root cortex of *N. amazonum*. In this species, lacunae were not observed; instead, only usual, but large intercellular spaces, which are able to mediate gas transport, were seen. Possibly, the root-shoot junction blocks oxygen transport from the atmosphere via the shoot to the roots. In *N. amazonum*, GRAFFMANN (2000) found a very low rate of transport of a tracer gas from the stem basis to the roots - only one fourth of the rate measured in *P. munguba*. Small trees of *N. amazonum* developed adventitious roots after 5 weeks during a flooding experiment carried out in that study. In the present study, oxygen could not be measured in adventitious roots, which possibly can be oxygenated, because the young cuttings in our experiments did not form such roots during stagnant cultivation.

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It should be noted that in this study only small cuttings with very young roots were examined. Older roots, after prolonged growth in anoxic medium, as well as newly developed adventitious roots may exhibit better-developed aerenchyma, resulting in measurable or better oxygenation. Cortex oxygen concentrations in adventitious roots of *S. martiana* that exhibit mature aerenchyma tissue that can be compared with four tubes, exceeded those in *P. munguba* roots (DE SIMONE et al. 2002). The moderate oxygenation of *T. pyramidalis* roots was comparable to that of the adventitious roots of *T. juruana*, the cortex of which lacks aerenchyma but has similar widened intercellular spaces (DE SIMONE et al. 2002). In this context, it must be emphasized that the roots of *T. pyramidalis* and some roots of *P. munguba* had been aerobically grown but nonetheless remained vital in anoxic medium and were able to transport oxygen. This finding suggests that atmospheric oxygen can diffuse both in newly formed adventitious roots and in porous original roots. DE SIMONE et al. (2002) exclusively investigated porous adventitious roots that had been grown in agar under anoxic conditions. Since the original roots of *S. martiana* die, they cannot provide a long-term pathway for oxygen transport (JUNK personal communication). Lacunae are constitutively formed in *T. pyramidalis* and *P. munguba* and may quickly enlarge in response to anoxia. In both species, aerenchyma formation seems to require the death of parenchymatic cells (lysigenous aerenchyma) (JACKSON & ARMSTRONG 1999; VOESENEK et al. 2006). In *P. munguba*; the remaining ligaments of the cell walls may act as stabilizing elements in the root cortex.

Suberin incrustations in the hypodermal cell layer constitute an apoplastic barrier to water uptake (ZIMMERMANN et al. 2000) and to oxygen diffusion from the root to the rhizosphere (DE SIMONE et al. 2003). ARMSTRONG et al. (2000) proposed that lignification is the anatomic basis of those barriers, but suberin deposits could also contribute to the low permeability of the hypodermal layers. Knowledge regarding the specific contribution of lignin and suberin to barrier function is scant. Oxygen measurements in and around agar-embedded roots together with the visualization of suberin deposits in the hypodermis by fluorescence microscopy indicated that suberin plays the most important role in limiting gas diffusion (DE SIMONE et al. 2002). In the present study, oxygen measurements and fluorescence microscopy together provided information about oxygen transport to the roots, its maintenance in the roots and its loss to the rhizosphere. In *T. pyramidalis*, the suberin barrier maintains cortical oxygen derived from internal transport for metabolism, as evidenced by root elongation. For *T. pyramidalis* the barrier seems to be of particular importance since the irregularly arranged lacunae do not permit highly effective transport. COLMER et al. (1998) and MCDO-NALD et al. (2001) proposed enhanced longitudinal oxygen diffusion, which would allow greater penetration of the roots into the soil, in response to a hypodermal barrier that reduced oxygen loss as well. Another benefit of the apoplastic barrier may be the decreased influx of potentially toxic substances, for instance, $Fe^{2+}$, $Mn^{2+}$, and sulfide, and pathogens (COLMER et al. 1998). Clearly, the moderate amounts of suberin incrustations present in the non-oxygenated roots of *N. amazonum* suit this purpose.

The lack of suberin incrustations in the hypodermis of the roots of *P. munguba* allowed massive radial oxygen diffusion from the well-oxygenated cortex to the anoxic rhizosphere along the entire lengths of the roots. In spite of this massive loss, cortex oxygen concentrations were high and enabled fast elongation and deep penetration of the roots. The oxidation of reduced toxic compounds by lost oxygen creates a protective

layer around the roots. This survival strategy was even more pronounced in *S. martiana*. Previous laboratory experiments showed oxygenated layers of up to 1.5 cm wide around the roots of this Amazonian species (DE SIMONE et al. 2002). Microelectrode measurements also showed oxygen in the layers outside the roots that contributed to the oxygen profiles in the rhizosphere of maize (ARMSTRONG et al. 1994), rice (REVSBECH et al. 1999), and *Phragmites australis* (ARMSTRONG et al. 2000). However, most wetland species have a strong barrier to radial oxygen loss in their basal regions and thus release oxygen only in the vicinity of the root apex as *T. pyramidalis* (ARMSTRONG et al. 2000; COLMER 2003; CONNELL et al. 1999; DITTERT et al. 2006; McDONALD et al. 2002). The only exception seems to be the wetland species *Phalaris aquatica*, in which the adventitious roots exhibit radial oxygen loss (McDONALD et al. 2002). To our knowledge, the only woody species that lacks a barrier to radial oxygen loss in its adventitious roots is the Amazonian tree species *S. martiana* (DE SIMONE et al. 2003, 2002). Examinations of several tree species inhabiting the Amazonian inundation forest suggested that only those plants with very high amounts of oxygen in their root cortices ($\geq 3$ mg $l^{-1}$, *S. martiana* and *P. munguba*), such as accumulate by massive internal oxygen transport, afford the lack of apoplastic barriers in their hypodermis and thus have a positive influence on the redox potential of their rhizosphere.

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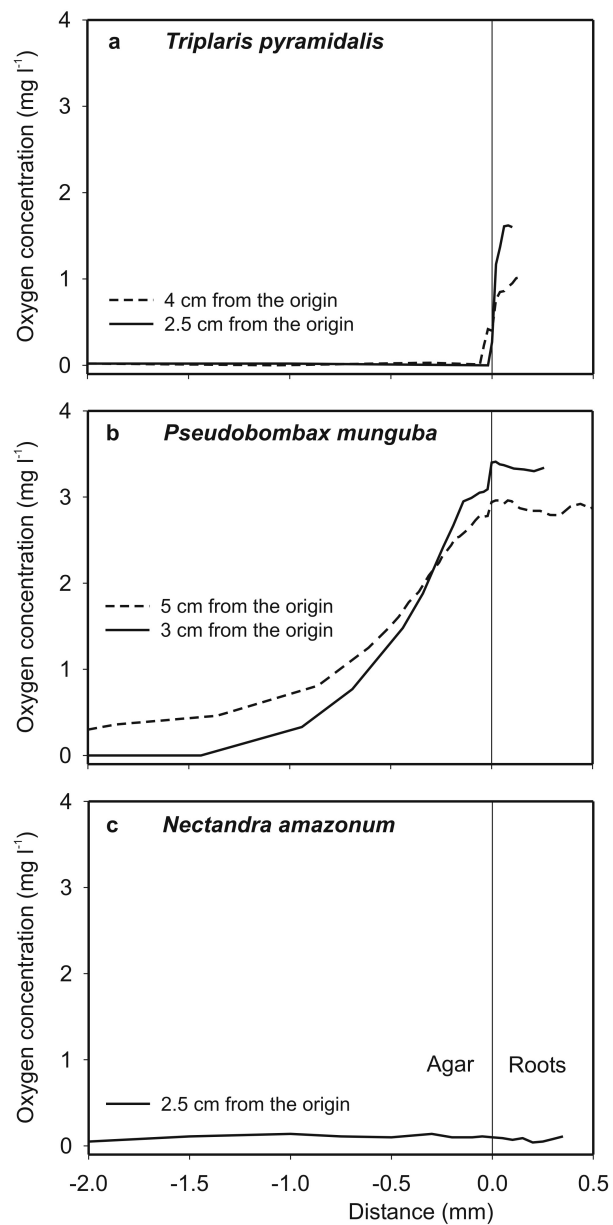
Fig. 1:

Typical oxygen profiles in the rhizosphere, exodermis, and outer cortex of the roots of the examined tree species. The vertical axis (distance 0 mm) indicates the root surface. (a) 5-cm-long original root of *T. pyramidales*. (b) 6-cm-long new root of *P. munguba*. (c) 6- to 7-cm-long original root of *N. amazonum*.
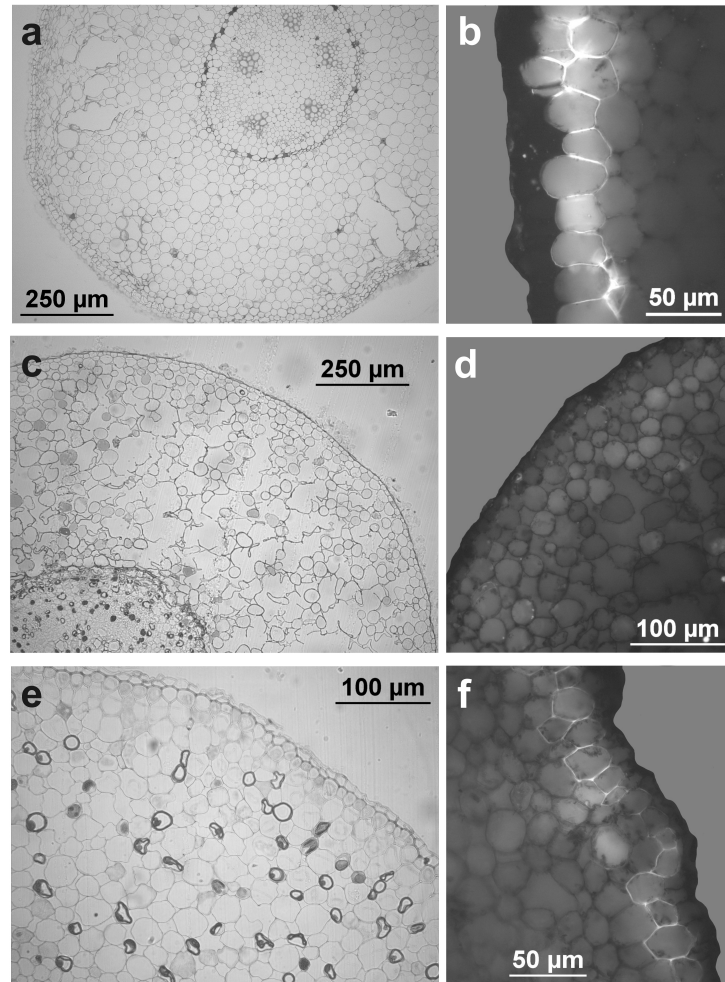
Fig. 2:

Typical transverse sections of roots harvested after 10 weeks of cultivation in stagnant agar nutrient solution. Cross-sections were taken ~1 cm behind the root apex. (a) Microtome-cut cross-section of a slowly growing original root of *T. pyramidalis* (5 cm long) stained with toluidine blue and viewed with light-field microscopy. (b) Free-hand-cut section of the same root of *T. pyramidalis*. Suberin staining revealed a strongly suberized hypodermis. (c) Microtome-cut cross-section of a new root of *P. munguba* (6-7 cm long) stained with toluidine blue and viewed with light-field microscopy. (d) Free-hand-cut section of the same root of *P. munguba*. Suberin staining revealed no suberization of the hypodermis. (e) Microtome-cut cross-section of an original root of *N. amazonum* (6-7 cm long) stained with toluidine blue and viewed with light-field microscopy. (f) Free-hand-cut section of an original root of *N. amazonum*. Suberin staining revealed a moderately suberized hypodermis.

Table 1: Growth rate, cortex oxygen concentration (mean value, measured 2-3 cm from the root origin), and number of comparable measurements and examined roots.

| Species | Growth rate (mm/d) | | Cortex oxygen concentration | | Number of of oxygen measurements* | Number of examined roots |
|---|---|---|---|---|---|---|
| | Original roots | Adventitious roots | (mg/l) | (hPa) | | |
| *T. pyramidalis* | 2 | n.d. | 1.5 | 3.8 | 8 | 4 |
| *P. munguba* | 5 | 5-10 | 3.4 | 8.6 | 8 | 4 |
| *N. amazonum* | 0 | n.d. | 0 | 0 | 10 | 5 |

*Comparable measuring points in each case 2-3 cm from the root origin.

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