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Stable carbon and nitrogen isotope compositions change with leaf age in two mangrove ferns

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ABSTRACT

After assimilation, plants often fractionate stable carbon and nitrogen isotopes among different organs depending on synthesis and transport of metabolites. We investigated stable carbon and nitrogen isotope compositions among leaves of different age (0 to 6 months) in two mangrove fern species (*Acrostichum danaeifolium* Langsd. & Fisch. and *Acrostichum aureum* L.) from Mexico. Leaves of all ages were analysed for $\delta^{13}C$, $\delta^{15}N$, carbon and nitrogen concentrations and gas exchange parameters. In both species, $\delta^{13}C$ slightly decreased with leaf age. Leaf salt concentration increased with leaf age, and thus did not decrease ^{13}C discrimination markedly. Enrichment in ^{13}C in younger *versus* older leaves can be explained by stage of development: Carbon is assimilated and incorporated into autotrophic leaves, but also transported as ^{13}C-enriched carbohydrates into still expanding and more heterotrophic younger leaves—indicated by lower rates of photosynthesis. Depletion in ^{13}C in old autotrophic leaves, which export photosynthetic assimilates, could mainly be explained by respiratory fractionation releasing ^{13}C-enriched CO_2. In contrast, $\delta^{15}N$ values in *A. danaeifolium* increased with leaf age. This pattern could be related to the transport of ^{15}N-depleted amino acids into younger leaves, and leaf construction with these compounds. In conclusion, ^{13}C depletion and ^{15}N enrichment with leaf age were described for other plant species earlier and were explained by different mechanisms of carbon and nitrogen assimilation and the export of these assimilates from older to younger leaves. These stable isotope patterns were approved for two mangrove fern species in this study.

Introduction

Fractionation of ^{13}C during photosynthesis in plants with the C_3 photosynthetic pathway (Calvin–Benson cycle) results in $\delta^{13}C$ values between −22 and −32‰ with an average of −27‰ (Boutton, 1996; Farquhar et al., 1989; O'Leary, 1981). Once plants have assimilated carbon, further ^{13}C fractionation processes take place. These are caused by equilibrium reactions or kinetic processes catalysed by specific enzymes (O'Leary, 1981), or by fractionation associated with transport of metabolites across organ boundaries or by fractionation during heterotrophic metabolism (Badeck et al., 2005). In the latter, isotope fractionation may occur either due to compartmentation of the source organ or due to a metabolic branching point or during the export process itself (Badeck et al., 2005).

Within plants, $\delta^{13}C$ values of different compounds vary. It has been observed that lignin and lipids are usually depleted in ^{13}C compared to the bulk plant material, while sugars, amino acids, and hemicelluloses are enriched in ^{13}C (Boutton, 1996; Bowling et al., 2008; Hobbie and Werner, 2004; Wiesenberg et al., 2004, 2008). The specific enrichment of ^{13}C often observed in transport compounds like sucrose leads to an enrichment of ^{13}C in the roots (Hobbie and Werner, 2004). On average, roots of C_3 plants have been shown to be enriched in ^{13}C by +1.2‰ compared to whole shoots (Werth and Kuzyakov, 2010) and by +2.3‰ compared to leaves (Bowling et al., 2008).

Significant $\delta^{13}C$ variation among different plant organs has been demonstrated for autotrophic shoots and leaves *versus* roots as well as among other heterotrophic plant organs, like young leaves, stems, flowers, fruits, and seeds (Badeck et al., 2005; Bathellier et al., 2008; Bowling et al., 2008; Cernusak et al., 2005; Damesin et al., 1998; Damesin and Lelarge, 2003; Ghashghaie and Badeck, 2014; Lamade et al., 2009; O'Leary, 1981). Leaf age is of special interest for studying isotope fractionation, because younger leaves show different physiological properties

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compared to older leaves (Medina et al., 2010; Terwilliger et al., 2001).

Whereas carbon is directly assimilated from the air or remobilized from reserve carbohydrates, nitrogen as key nutrient to build up the photosynthetic apparatus is translocated either from the roots, storage organs or mature leaves to growing leaves. Studies on ^{15}N natural abundance among differently aged leaves are scarce, but Yoneyama and Kaneko (1989) have shown that $\delta^{15}N$ in roots was lower than in leaves of *Brassica campestris* plants. They explained this low $\delta^{15}N$ in the roots by a supply of ^{15}N-depleted amino acids from the shoots or by an efflux of ^{15}N-enriched nitrate to the shoots. Exudation of ^{15}N-enriched rhizodeposits could be a further explanation.

Stable carbon and nitrogen isotope compositions of different plant organs have been mainly studied for cultivated crops and trees; however, there is still a need to confirm these findings in a number of wild plant species (Badeck et al., 2005; Yoneyama et al., 2003). Therefore, we chose two mangrove ferns (*Acrostichum danaeifolium* Langsd. & Fisch. and *Acrostichum aureum* L.) to study ^{13}C and ^{15}N natural abundances in autotrophic and heterotrophic plant organs. Mangrove ferns are known to have a continuous leaf production in a spiral sequence which makes them suitable to study isotope fractionation among developing young leaves and fully-developed old leaves. It can be expected that expanding leaves are heterotrophic and import C and N from fully-expanded autotrophic leaves (Gary et al., 1998; Shishido et al., 1999). At the study site, *A. danaeifolium* leaves are developed continuously at a rate of 14.6 ± 0.44 leaves per year and have a mean life span of 7.7 months (Mehltreter and Palacios-Rios, 2003). While *A. danaeifolium* is a neotropical species, *A. aureum* is distributed pan-tropically. Both species grow at the study site and follow the same leaf phenology pattern (K. Mehltreter, pers. obs.).

A typical feature of mangrove soils is the high salt concentration which induces stress to plants growing on the site. *Acrostichum* species can tolerate these conditions by increasing salt and sugar concentrations in their tissues (Medina et al., 1990). When plants are exposed to high salinity, stomatal closure is induced by physiological drought (Munns and Tester, 2008), gas exchange of the leaves is reduced (Rawson et al., 1988), and leaf length and leaf life span decrease (Sharpe, 2010). Additionally, leaf Na^+ and $C1^-$ concentrations increase with leaf age in plants grown at increased salt concentrations (Greenway et al., 1965) possibly because older leaves have transpired for longer time (Rawson et al., 1988). As a result, we expected that increasing amounts of salt from younger to older leaves could cause a reduction in gas exchange parameters, especially a decrease in stomatal conductance and c_i, and consequently lead to decreasing ^{13}C discrimination and increasing $\delta^{13}C$ values according to Farquhar et al. (1989).

In this study we addressed the following questions: (1) Do $\delta^{13}C$ values of *A. danaeifolium* and *A. aureum* leaves decrease with leaf age due to development from heterotrophic to autotrophic organs? (2) Do $\delta^{13}C$ values increase with increasing sodium accumulation in plant leaves as an indicator of salt stress? (3) Do $\delta^{15}N$ values show a converse pattern to $\delta^{13}C$, *i.e.* do they increase with leaf age due to metabolic processes and translocation of ^{15}N-depleted compounds?

Materials and methods

Site description

The study was performed at the Biological Station of La Mancha (19° 36′ 00″ N, 96° 22′ 40″ W), Veracruz, Mexico. Studied plants were located at 100 m distance maximum to a brackish-water lagoon, in the shady understorey of a forest dominated by black mangroves (*Avicennia germinans* (L.) Stearn, Avicenniaceae) and red mangroves (*Rhizophora mangle* L., Rhizophoraceae) as a second minor contributor. The water level at the study site fluctuates little; the soils are always waterlogged. Climatic conditions are hot and humid, with a dry season from November to May, when mean precipitation is less than 45 mm per month. During the winter (December to March) north winds help to close the lagoon and disconnect it from the Gulf of Mexico. The water level in the lagoon rises when the lagoon's mouth is closed. Thus, the dry season in La Mancha coincides with the highest level of the water table and increased flooding of mangroves and wetlands. Mean annual temperature was 25.8 °C and mean annual precipitation was 1193 mm for the period between January 1981 and December 2009 (data collected from the Climatological Station of La Mancha, Comision Nacional de Agua, Servicio Meteorológico Nacional, http://smn.cna.gob.mx/climatologia/Mensuales/ver/00030353.TXT).

Measurements and samplings

Six large, healthy plants of *A. danaeifolium* and *A. aureum* were selected within an undisturbed mangrove forest fragment of about 1 ha size. Each leaf was tagged and its approximate age was assigned according to its position on the erect fern rhizome, assuming a leaf emergence rate of about one leaf per month (Mehltreter and Palacios-Rios, 2003). Thus the youngest developing leaf crozier was assigned age 0, the next still expanding leaf located at a circular distance of about 120 degrees (clock or counter clockwise) was assigned age 1 and each successive leaf was numbered consecutively up to the approximate age of 6 months.

On March 26, 2009, the net assimilation rate (A), the stomatal conductance (g_s), and the ratio of intercellular to atmospheric CO_2 concentration (c_i/c_a) were determined with a portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln, Nebraska, USA) for three individuals (samples 1, 2, and 3) of each species. Gas exchange was measured at a photosynthetic photon flux density (PPFD) of 100 $\mu mol\, m^{-2}\, s^{-1}$, a block temperature of the LI-6400 of 31.5 ± 1.2 °C, and a relative air humidity of 56.2 ± 5.7%, corresponding to the general microclimatic conditions in the mangrove understorey.

After the gas exchange measurements, we sampled about ten leaflets of each leaf and each of the 12 study plants, brought them immediately to the laboratory of the biological station and dried them in an oven for 72 h at 60 °C. In Germany, dry leaf samples were ground with an ultra-centrifugal mill (Retsch, Haan, Germany) with an insert of 0.25 mm mesh size. After grinding of each leaf sample, the mill was cleaned from remaining powder with compressed air, and after each species and each leaf age, the mill was wiped out with ethanol (70%).

Elemental and isotope analyses

For carbon (C), nitrogen (N), and isotope analyses, 0.7 mg ground sample material was filled into tin capsules (4 mm × 6 mm, IVA Analysentechnik, Meerbusch, Germany). Analyses were performed at an elemental analyser (Euro EA C/N analyser, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (Thermo Finnigan Delta plus Advantage, Thermo Fisher Scientific Inc., Waltham, USA). Laboratory reference gases (CO_2 and N_2) calibrated against USGS40 were used to obtain $\delta^{13}C$ and $\delta^{15}N$ values (Qi et al., 2003). Acetanilide was used as a laboratory standard for isotopic and elemental drift control. Isotopic differences $\Delta^{13}C_{reference}$ or $\Delta^{15}N_{reference}$ among plant organs or between chemical compounds and source plant organs cited from literature in the introduction