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Original Article

Experimental evidence for diel $\delta^{15}N$-patterns in different tissues, xylem and phloem saps of castor bean (*Ricinus communis* L.)

A. D. PEUKE[1,2], A. GESSLER[2,3] & G. TCHERKEZ[4,5]

[1]ADP International Plant Science Consulting, Talstraße 8, 79194 Gundelfingen, Germany, [2]Institute for Landscape Biogeochemistry, Leibniz-Centre for Agricultural Landscape Research (ZALF) e.V., Eberswalder Strasse. 84, 15374 Müncheberg, Germany, [3]Professorship for Landscape Biogeochemistry, Faculty of Agriculture and Horticulture, Humboldt University at Berlin, Lentze-Allee 75, 14195 Berlin, Germany, [4]Institut de Biologie des Plantes, CNRS UMR 8618, Université Paris-Sud 11, 91405 Orsay Cedex, France and [5]Institut Universitaire de France, 103 boulevard Saint-Michel, 75005 Paris, France

ABSTRACT

Nitrogen isotope signatures in plants might give insights in the metabolism and allocation of nitrogen. To obtain a deeper understanding of the modifications of the nitrogen isotope signatures, we determined $\delta^{15}N$ in transport saps and in different fractions of leaves, axes and roots during a diel course along the plant axis. The most significant diel variations were observed in xylem and phloem saps where $\delta^{15}N$ was significantly higher during the day compared with during the night. However in xylem saps, this was observed only in the canopy, but not at the hypocotyl positions. In the canopy, $\delta^{15}N$ was correlated fairly well between phloem and xylem saps. These variations in $\delta^{15}N$ in transport saps can be attributed to nitrate reduction in leaves during the photoperiod as well as to ^{15}N-enriched glutamine acting as transport form of N. $\delta^{15}N$ of the water soluble fraction of roots and leaves partially affected $\delta^{15}N$ of phloem and xylems saps. $\delta^{15}N$ patterns are likely the result of a complex set of interactions and N-fluxes between plant organs. Furthermore, the natural nitrogen isotope abundance in plant tissue is not constant during the diel course – a fact that needs to be taken into account when sampling for isotopic studies.

Key-words: day/night cycle; isotope fractionation; nitrogen; transport.

INTRODUCTION

Nitrogen isotopes are well-recognized tools in plant physiology and eco(physio)logy (Högberg 1997; Robinson 2001; Dawson *et al.* 2002). The natural nitrogen isotope composition ($\delta^{15}N$) is now being widely used in research on N cycling in organisms and ecosystems. ^{15}N natural abundances are used in fundamentally different ways, from traditional ^{15}N tracers by integrating N cycle processes via N isotope fractionations and the mixing of various N-containing pools (Robinson 2001). Nitrogen isotope composition of plant material is determined by the isotope ratio of the external nitrogen source (nitrate, ammonium, amino acids and/or N_2) and physiological mechanisms within the plant like assimilation events, loss of nitrogen, resorption and reallocation of nitrogen (Högberg 1997; Robinson, Handley & Scrimgeour 1998; Comstock 2001; Evans 2001; Robinson 2001; Dawson *et al.* 2002; Werner & Schmidt 2002; Craine *et al.* 2009). The bulk nitrogen pool of plant organs contains multiple N species such as inorganic nitrogen, amino acids, proteins and chlorophylls. Variations in $\delta^{15}N$ can thus also be attributed to different mixing ratios of different N species, each of which could potentially have a distinct $\delta^{15}N$ (Werner & Schmidt 2002; Tcherkez 2011; Gauthier *et al.* 2012). Consequently, there is substantial variation in $\delta^{15}N$ values between ecosystems, plant species, plant individuals or plant parts and biochemical fractions. Handley *et al.* (1999) and Craine *et al.* (2009) reported that foliar $\delta^{15}N$ increased with decreasing mean annual precipitation and with increasing mean annual temperature. The variation range in plant $\delta^{15}N$ is generally –10 to +10‰ (Evans 2001) with quite large differences between plants with nitrate as the sole nitrogen source (Högberg 1997). It is believed that nitrate availability contributes to this variability, plant cultivated under low nitrate concentration being less depleted in ^{15}N than those cultivated under higher nitrate supply (Evans 2001). In case of low nitrate concentration in the soil, the efflux of (^{15}N-enriched) nitrate from the root to the soil is limited, and as a result, all nitrate taken up will be assimilated. Additionally, at higher nitrate supply, more nitrate is transported to the shoots for assimilation (Peuke *et al.* 1996), which may affect the $\delta^{15}N$ of the shoots. Furthermore, there are differences in $\delta^{15}N$ between plant organs, reported both in the lab and in the field (Högberg 1997), with shoots being generally ^{15}N-enriched compared with roots (Yoneyama *et al.* 1997; Peuke, Gessler & Rennenberg 2006). It is believed that this difference in $\delta^{15}N$ value is caused by the isotope fractionation against ^{15}N during nitrate reduction: nitrate molecules left behind after reduction in roots are ^{15}N-enriched and translocated to shoots (for a review, see Tcherkez & Hodges 2008). As a matter of fact, the allocation of nitrogenous compounds of contrasted $\delta^{15}N$ is thus certainly the cornerstone causing differences in $\delta^{15}N$ between plant parts.

Correspondence: A. D. Peuke. Fax: (0)3212 1137807; e-mail: andreas@peuke.de, http://www.peuke.de