Plant Ecology and Evolution 149 (1): 39–44, 2016
http://dx.doi.org/10.5091/plecevo.2016.1112

REGULAR PAPER

Tracing nitrogen flow in a root-hemiparasitic association by foliar stable-isotope labelling

Petra Světlíková, Petr Blažek, Radka Mühlsteinová & Jakub Těšitel*

Faculty of Science, University of South Bohemia, Branišovská 1760, České Budějovice CZ -370 05, Czech Republic
*Author for correspondence: jakub.tesitel@centrum.cz

Background and aims – The resource flows in the host-hemiparasite association have been frequently studied by applying stable isotope techniques. However, these methods of artificial labelling required sophisticated equipment preventing their application to field experiments. Here, we aimed to test the applicability of the $^{15}N^{13}C$-urea foliar brushing method in tracing the resource flows between a root hemiparasite, *Rhinanthus major*, and a host, *Triticum aestivum*. In addition, the dynamics of the label movement was examined in order to provide an estimate of the most appropriate harvesting time.
Methods – Double-labelled urea (98 atom % ^{15}N, 99 atom % ^{13}C) solution (2 g dm^{-3}) was applied on host plants grown with hemiparasites by a single foliar brushing. Above- and belowground biomass of both species was harvested 3, 7, and 14 d after host labelling and its isotopic composition was analyzed. Final isotopic enrichment of biomass was expressed as the atom percent difference between labelled samples and the mean of corresponding controls.
Key results – Our results showed that a single leaf-brushing with $^{15}N^{13}C$-urea provided sufficiently ^{15}N-labelled plant material, but it was insufficient to shift the natural abundance of ^{13}C in both species. Similar ^{15}N values were found for the host and hemiparasite biomass already 3 d after labelling, but the ^{15}N enrichment of attached hemiparasite significantly increased in time. Within a week, ^{15}N-label gradually dispersed into the host tissues and was simultaneously transferred into the hemiparasite via the root connections.
Conclusions – We present foliar brushing by ^{15}N-urea as a simple and precise labelling method, which can be widely applied in both greenhouse and field experiments to examine the nitrogen flows between root hemiparasites and their host species. The transfer of nitrogen to the hemiparasite is fast and thus an experimental period of 7 d seems largely sufficient for field studies where the equilibrium state of labelling is of interest.

Key words – Haustorium, leaf, nitrogen flow, Orobanchaceae, hemiparasitic plant, *Rhinanthus*, stable isotope, *Striga*, tracer.

INTRODUCTION

Root hemiparasites are parasitic plants that attach belowground to roots of other plants, withdrawing resources from host vascular bundles and performing their own photosynthesis at the same time (Press 1989). Root hemiparasitism is one of the most common life strategies among parasitic plants (Heide-Jørgensen 2008). Many root hemiparasites have been demonstrated to play important roles in the ecosystem by altering nutrient cycling (Press 1998, Quested et al. 2005, Bardgett et al. 2006, Demey et al. 2014) or changing competitive relations in plant communities (Gibson & Watkinson 1991, Pywell et al. 2004). Others, namely several species of the genus *Striga*, have been extremely harmful weeds causing enormous economical losses in dry tropical and subtropical regions (Parker 2009).

Physiology of the hemiparasite-host association is of central importance when studying the biology of root hemiparasites (Těšitel et al. 2015). The association basically involves two autotrophic plants connected by a unidirectional flow of resources (Jiang et al. 2003, 2004). Quantitative and qualitative analyses of this resource flow as well as the detection of its effect on the physiology of both partners have been the main goals of many physiological studies on root hemiparasites. The application of stable isotope techniques is a frequently used methodological approach in these physiological studies (Ducharme & Ehleringer 1996, Pageau et al. 1998, Pate & Bell 2000, Aflakpui et al. 2005, Cameron & Seel

2007, Těšitel et al. 2010). The use of isotope tracing techniques requires a contrast in stable isotopic composition of hosts and hemiparasites, which can be based either on their natural abundances, e.g. the use of C_4 hosts in the studies of carbon translocation (Ducharme & Ehleringer 1996, Pageau et al. 1998, Pate & Bell 2000, Santos-Izquierdo et al. 2008, Těšitel et al. 2010) or artificial labelling (Pageau et al. 2003, Aflakpui et al. 2005, Cameron & Seel 2007).

Isotope labelling of the host plant is the first step of any study using artificial labelling. Various methods can be used to produce plants enriched in ^{15}N and/or ^{13}C stable isotopes. However, the vast majority of these methods require sophisticated equipment comprising gas-tight chambers and other system components necessary for precise labelling. Moreover, different ^{15}N labelling methods can vary in their effectiveness and depend on a focal species (Hertenberger & Wanek 2004). A relatively new and much more feasible method for in situ ^{15}N and ^{13}C labelling of plants is based on foliar feeding of plants with a double-labelled urea solution. This method was firstly introduced by Schmidt & Scrimgeour (2001), who simultaneously enriched a plant tissue in N and C stable isotopes by daily foliar misting, extending the application of the method to C translocation studies. The method was later modified by Putz et al. (2011), who replaced foliar misting by brushing, which prevents the contamination of soil and co-occurring plants. Leaf-brushing by a double-labelled urea solution has been suggested as a straightforward, low-cost and technically easy way of controlled isotope labelling of plants.

In addition to the leaf-misting and leaf-brushing labelling methods, another feasible labelling method has been widely used both in greenhouse and field to study nutrient flow between plants. This method, developed by Ledgard et al. (1985), introduces ^{15}N by immersion of a leaf in a ^{15}N-enriched urea solution and mostly serves to detect and further examine nitrogen transfer between legumes and neighbouring plant species (Ledgard et al. 1985, McNeill et al. 1997, Gylfadóttir et al. 2007, Pirhofer-Walzl et al. 2012) and below-ground N deposition from legumes in the soil (McNeill et al. 1997, Hertenberger & Wanek 2004, Gasser et al. 2015).

Here, we tested the applicability of the $^{15}N^{13}C$-urea foliar brushing method in tracing the resource flows between a host and root hemiparasite. Not only did we aim to demonstrate the flow of nitrogen and carbon, but also the dynamics of the label movement. This is crucial for practical use as it provides a guideline regarding the length of the period between labelling and sampling for stable isotope analysis. The study used a model root-hemiparasitic association between hemiparasitic *Rhinanthus major* L. (= *R. angustifolius, R. serotinus*; Orobanchaceae) and a host, *Triticum aestivum* L. (common wheat).

METHODS

Cultivation and stable isotope analysis

Rhinanthus major seeds were collected from a natural population occurring on the Čertoryje meadows, Bílé Karpaty Mts., Czech Republic. Seeds of common wheat were obtained from the school farm of the Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic.

Seeds of *Rhinanthus* were germinated for 86 days on Petri dishes padded with moist filter paper at 4°C to break seed dormancy. Seeds of common wheat were germinated on Petri dishes with moist filter paper for four days at room temperature. Seedlings of both the parasite and host were planted to 0.8 dm^3 pots filled with a 1:1 (v/v) mixture of universal gardening compost and sand. The distance between the parasite and host was 3–4 cm. Plants were cultivated in a growth chamber at the Department of Botany, Faculty of Science, University of South Bohemia, Czech Republic under following conditions: 12:12 h light:dark cycle, PAR intensity 400–500 µmol m^{-2} s^{-1}, and 23°C/18°C day/night temperature.

Labelling of host by $^{15}N^{13}C$-urea (98 atom % ^{15}N, 99 atom % ^{13}C; obtained from Sigma-Aldrich Corporation, St. Louis, USA) was conducted after 44 days of growth. The concentration of double-labelled urea in the labelling solution was 2 g dm^{-3}, which corresponds to 62.2 mmol ^{15}N dm^{-3} and 31.1 mmol ^{13}C dm^{-3}. The labelling solution and a drop of detergent were applied by brush on 5-cm long sections of host leaves (3 leaves per host plant). The labelled sections were marked by paper stickers for a permanent identification

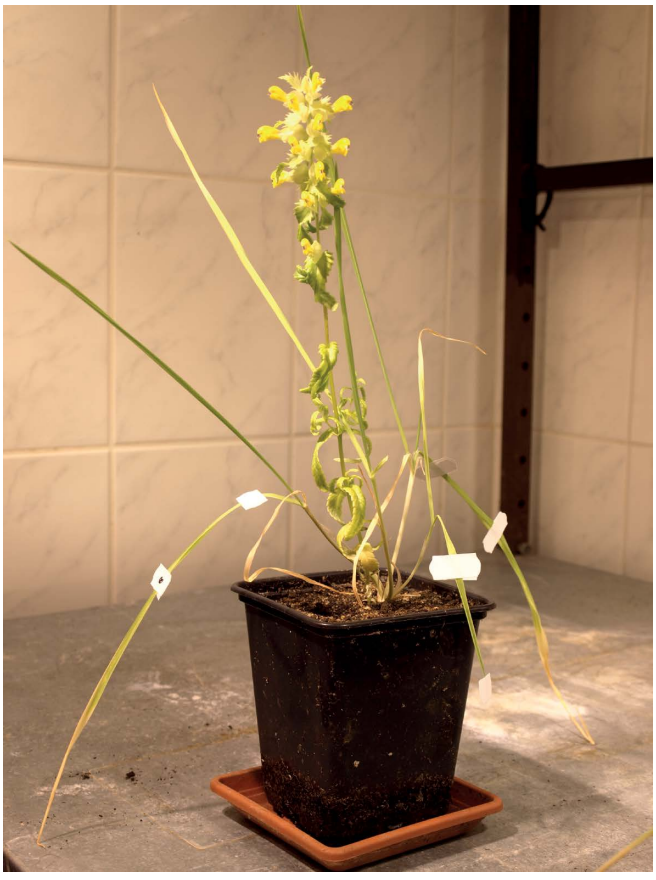

Figure 1 – $^{15}N^{13}C$-urea labelled host plant, *Triticum aestivum*, and attached hemiparasite, *Rhinanthus major*. Note the paper stickers used to mark the leaf sections where the labelling solution was applied. The picture was taken at the time of harvest (14 d after labelling).

Table 1 – Analysis of variance table of linear models.
The table summarizes the effects of time, plant part (shoot vs. root), and their interaction on the ^{15}N atom percent excess in the biomass of $^{15}N^{13}C$-urea labelled hosts (unlabelled sections) and attached hemiparasites. Significant effects ($p < 0.05$) are marked in bold.

Effect		Hemiparasite			Host		
	DF	SS	F	p	SS	F	p
Time of harvest	1	**0.00714**	**5.638**	**0.024**	0.00024	0.075	0.786
Plant part	1	0.00018	0.143	0.708	0.00639	2.020	0.166
Time × Part	1	0.00072	0.565	0.458	0.00057	0.180	0.675
Residuals	30	0.03799			0.09419		

(fig. 1). There were seventeen labelled pots and nine unlabelled control pots in total. The pots were positioned at random in the growth chamber at a distance to prevent contact between plants in different pots.

Five to seven experimental and three control pots were harvested 3, 7, and 14 d after host labelling. Above- and below-ground biomass samples of each host and hemiparasite were collected in separate paper bags and dried at 80°C for 48 hours. The host leaf sections on which the labelling solution was applied were processed separately. Dried biomass was homogenized and a subset of it was embedded in tin capsules for stable isotope analysis.

The stable isotope analysis was conducted with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility at UC Davis (University of California, Davis, CA, USA). The N and C isotopic compositions of the biomass samples was expressed as ^{15}N and ^{13}C atom percent relative to the international standards, Air and V-PDB (Vienna PeeDee Belemnite), respectively.

Data analyses

The ^{15}N and ^{13}C atom percent data of each sample type (host/hemiparasite, root/shoot biomass, harvesting time, labelled/control pots) were plotted as boxplots to illustrate the isotopic composition of the samples. Since a substantial enrichment of the experimental pots in heavy isotopes was observed only for ^{15}N, the ^{13}C data were not further analyzed. ^{15}N atom percent excess was calculated by subtracting the mean atom percent of corresponding control sample from each labelled sample value. Linear models were used to test the effect of time (days after labelling), plant part (shoot vs. root), and their interaction on the ^{15}N atom percent excess of the labelled pot samples of host and parasite separately. All statistical analyses were conducted in R, version 3.0.1 (R Core Team 2013).

RESULTS AND DISCUSSION

All samples from labelled pots were substantially enriched in ^{15}N compared to the controls and the ^{15}N isotopic composition of labelled and control pots did not overlap (fig. 2, electronic appendix 1). The samples from labelled pots largely varied in ^{15}N atom percent, while control pot samples showed almost no variation (electronic appendix 1). The parts of host leaves where the label had been applied were highly ^{15}N enriched 3 and 7 d after labelling when compared with non-labelled parts of host leaves, but this difference decreased after 14 d (electronic appendix 1). Similar ^{15}N values were found for the host and hemiparasite biomass already 3 d after labelling, but the enrichment in ^{15}N of *Rhinanthus* attached to a labelled host significantly increased in time ($F_{1, 30} = 5.64$, $p = 0.024$; table 1, fig. 2). The ^{15}N enrichment further increased between 3 and 7 d after labelling, but not between 7 and 14 d, suggesting that the major transfer of the label to the hemiparasite occurred during the week after host labelling (fig. 2). We found a similar pattern for the host biomass, but

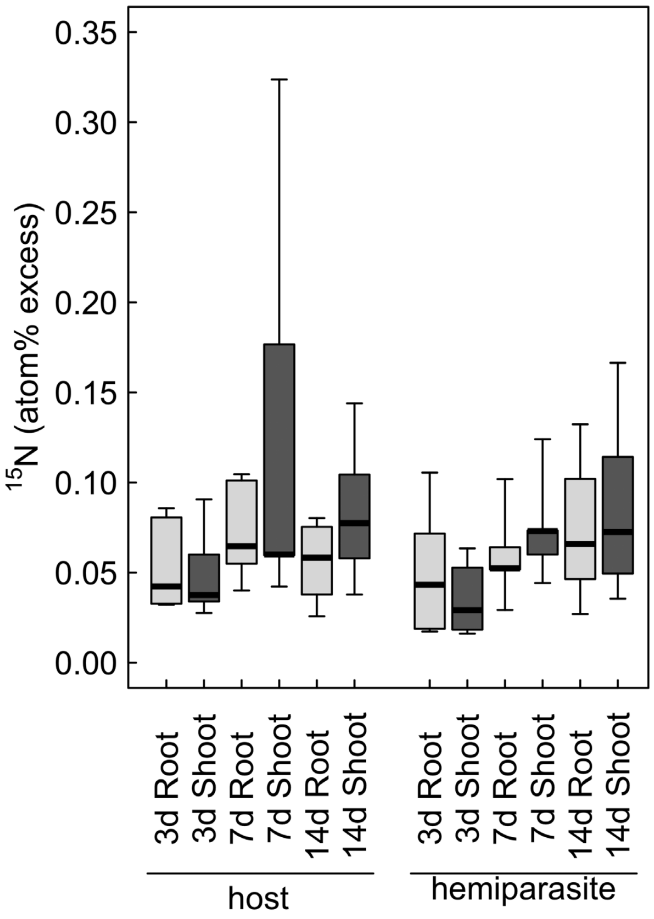

Figure 2 – Distribution of ^{15}N atom percent excess in roots and unlabelled sections of shoots of the hemiparasite, *Rhinanthus major*, and the host, *Triticum aestivum*, harvested 3, 7, and 14 d after host labelling. Medians, quartiles, and ranges are displayed. $n = 7$ for labelled samples collected on day 14, $n = 5$ for other labelled samples, and $n = 3$ for control samples.

its increase in time was not significant (table 1) which was probably caused by great variation in ^{15}N of host shoots 7 d after labelling (fig. 2). These results indicate a gradual translocation of the label into the host tissue and its immediate transfer into the hemiparasite via the root connections. Consequently, the harvesting period should be shifted to earlier dates, e.g. 1 to 7 d instead of 3 to 14 d, in order to examine the dynamics of the label movement in the host-hemiparasite association in more detail. However, an experimental period of 7 d seems largely sufficient for field studies where the major transfer of the label is of interest.

None of the other tested effects comprising hemiparasite plant material and its interaction with time significantly differed in ^{15}N atom percent (table 1). Moreover, ^{15}N atom percent of the host was not significantly affected by any of the tested predictors (table 1). This is in contrast with other studies reporting lower isotopic enrichment in roots due to the preferential storage of absorbed N in shoots (Below et al. 1985, Ledgard et al. 1985, McNeill et al. 1997, Schmidt & Scrimgeour 2001, Putz et al. 2011). The hemiparasite may alter this relationship in host species by supporting the preferential translocation of absorbed tracer to host roots, from which it is acquired by the hemiparasite resulting in no significant differences in the tracer between host roots and shoots. However, a comparison with non-infected hosts would be needed to confirm such a possibility.

In contrast to ^{15}N, the samples of labelled and control pots displayed very small differences in ^{13}C atom percent (appendix 2). Isotope labelling had a significant effect on ^{13}C composition of the host and hemiparasite tissues 3 d after labelling ($F_{1, 13} = 10.61$, $p = 0.006$; $F_{1, 13} = 24.41$, $p = 0.0003$, respectively). This initial enrichment diminished already 7d after labelling which was clearly caused by the dilution of the labelled carbon by newly produced assimilates. Additionally, roots of the hemiparasite had significantly higher ^{13}C atom percent than its shoots ($F_{1,13} = 109.6$, $p < 0.0001$). Despite being statistically significant, the absolute size of the differences was too small to be interpreted or further discussed. However, the shift in ^{13}C composition of the hemiparasite following host labelling presents a qualitative evidence on the uptake of host-derived carbon by root-hemiparasitic plants. As such it complements the previous studies based on radioisotope tracing (e.g. Govier et al. 1967), natural abundance of carbon stable isotope (e.g. Press et al. 1987, Těšitel et al. 2010), and composition analyses of simultaneously collected host and hemiparasite xylem sap (e.g. Seel & Jeschke 1999).

The lower enrichment of plants in ^{13}C than in ^{15}N was also found in other studies using leaf-brushing or spraying labelling with $^{15}N^{13}C$-urea solution (Schmidt & Scrimgeour 2001, Putz et al. 2011). According to Putz et al. (2011), it might be a consequence of the atomic structure of urea containing two atoms of N per one atom of C, and it might result from the loss of ^{13}C through respiration. Another reason for the lower enrichment in ^{13}C might be the N over-supply of the plant decreasing carbohydrate accumulation. We can exclude an exchange of ^{13}C between labelled and unlabelled plants by photorespiration and photosynthesis, as we did not detect this in a previous experiment (Těšitel et al. 2010).

Therefore, a single leaf-brushing labelling by a $^{15}N^{13}C$-urea provided sufficiently ^{15}N-labelled plant material. A single foliar application of ^{15}N-urea was also recently validated as a new method of studying seed dispersal and seedling recruitment (Castellano & Gorchov 2013). By contrast, the single brushing was insufficient for C labelling. The application of more concentrated $^{15}N^{13}C$-urea solution or repeated labelling might provide plants that are sufficiently enriched in both stable isotopes. That might, however, affect the plants by providing a significant N-supply. Repeated labelling by a low-concentrated urea solution would be more appropriate, as the application of more concentrated urea (> 5 g dm^{-3}) was shown to cause an N over-supply or leaf burning in crop plants (Hinsvark et al. 1953, Bremner 1995).

Experimental studies on hemiparasites frequently used ^{15}N isotope tracers to elucidate the host-hemiparasite nutrient translocation (Pageau et al. 2003, Cameron & Seel 2007) or the functional role of hemiparasites in ecosystems (Ameloot et al. 2008, Demey et al. 2013, 2014). However, urea/doubly-labelled urea leaf-feeding has never been employed as labelling method in these studies. To trace the resource transfer to the hemiparasite, Pageau et al. (2003) and Cameron & Seel (2007) subjected roots of the host species to a $K^{15}NO_3$ labelling solution. Although the method provided evidence about a non-specific transfer of nutrients through transpiration stream of *Striga* (Pageau et al. 2003) and high effectiveness of resistance mechanisms of two forb species (Cameron & Seel 2007), it required a highly sophisticated pot design, not applicable to field tracing experiments. In contrast, the field studies by Ameloot et al. (2008) and Demey et al. (2013) used spraying to apply ^{15}N labelling solution onto their experimental plots. Although this method is simple and provided a large amount of data on N turnover in the experimental communities, it is largely unspecific and cannot be used to study the individual host-hemiparasite interaction.

The leaf-immersion method might also be applied to examine nitrogen flows at the hemiparasite-host interface, although it seems to be not so simple and easy to do compared to leaf brushing. Similarly to other shoot-labelling techniques, even leaf-immersion can introduce some artifacts leading to over- or underestimation of transferred nitrogen (Pirhofer-Walzl et al. 2012, Chalk et al. 2014). For example, the direct leakage of applied label or transfer of the absorbed label to the soil result in the overestimation of transferred nitrogen (McNeill et al. 1997, Khan et al. 2002, Gylfadóttir et al. 2007). Thus caution must be taken when interpreting the results provided by shoot labelling.

Our results confirmed the applicability of single foliar brushing by $^{15}N^{13}C$-urea (of ^{15}N-urea) in tracing nitrogen flow between the host and root hemiparasite. Repeated labelling by low-concentrated urea would probably be necessary to track carbon flow in the host-hemiparasite association. The main advantage of the foliar brushing method is its simplicity on the one hand and specificity on the other hand. Thus, leaf brushing by ^{15}N-urea can be widely applied in both greenhouse and field experiments in order to examine the nitrogen flows between root hemiparasites and their various host species. If applied in the field, rainy conditions should be definitely avoided to prevent direct root uptake of the label. Using doubly labelled urea for monitoring both ni-

trogen and carbon would, however, require further optimization of the labelling protocol.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingenta-connect.com/content/botbel/plecevo/supp-data), and consist of: (1) ^{15}N atom percent in shoots and roots of the hemiparasite and host harvested 3, 7, and 14 d after host labelling by $^{15}N^{13}C$-urea in labelled and control pots; and (2) ^{13}C atom percent in shoots and roots of the hemiparasite and host harvested 3, 7, and 14 d after host labelling by $^{15}N^{13}C$-urea in labelled and control pots.

ACKNOWLEDGEMENTS

We would like to thank Jitka Neuwirthová and Bára Zdvihalová for their help with sample processing, and Andreas Demey and an anonymous reviewer for constructive comments that helped improve the manuscript. The study was funded by the Czech Science Foundation (project no. 14-26779P).

REFERENCES

Aflakpui G.K.S., Gregory P.J., Froud-Williams R.J. (2005) Carbon (^{13}C) and nitrogen (^{15}N) translocation in a maize-Striga hermonthica association. Experimental Agriculture 41: 321–333. http://dx.doi.org/10.1017/S0014479705002711

Ameloot E., Verlinden G., Boeckx P., Verheyen K., Hermy M. (2008) Impact of hemiparasitic Rhinanthus angustifolius and R. minor on nitrogen availability in grasslands. Plant and Soil 311: 255–268. http://dx.doi.org/10.1007/s11104-008-9640-2

Bardgett R.D., Smith R.S., Shiel R.S., Peacock S., Simkin J.M., Quirk H., Hobbs P.J. (2006) Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. Nature 439: 969–972. http://dx.doi.org/10.1038/nature04197

Below F.E., Crafts-Brandner S.J., Harper J.E., Hageman R.H. (1985) Uptake, distribution, and remobilization of ^{15}N-labeled urea applied to maize canopies. Agronomy Journal 77: 412–415. http://dx.doi.org/10.2134/agronj1985.00021962007700030014x

Bremner J.M. (1995) Recent research on problems in the use of urea as a nitrogen fertilizer. Fertilizer Research 42: 321–329. http://dx.doi.org/10.1007/BF00750524

Cameron D.D., Seel W.E. (2007) Functional anatomy of haustoria formed by Rhinanthus minor: linking evidence from histology and isotope tracing. New Phytologist 174: 412–419. http://dx.doi.org/10.1111/j.1469-8137.2007.02013.x

Castellano S.M., Gorchov D.L. (2013) Using a stable isotope to label seeds and seedlings of an invasive shrub, Lonicera maackii. Invasive Plant Science and Management 6: 112–117. http://dx.doi.org/10.1614/IPSM-D-12-00053.1

Chalk P.M., Peoples M.B., McNeill A.M., Boddey R.M., Unkovich M.J., Gardener M.J., Silva C.F., Chen D. (2014) Methodologies for estimating nitrogen transfer between legumes and companion species in agro-ecosystems: A review of ^{15}N-enriched techniques. Soil Biology and Biochemistry 73: 10–21. http://dx.doi.org/10.1016/j.soilbio.2014.02.005

Demey A., Staelens J., Baeten L., Boeckx P., Hermy M., Kattge J., Verheyen K. (2013) Nutrient input from hemiparasitic litter favors plant species with a fast-growth strategy. Plant and Soil 371: 53–66. http://dx.doi.org/10.1007/s11104-013-1658-4

Demey A., Rütting T., Huygens D., Staelens J., Hermy M., Verheyen K., Boeckx P. (2014) Hemiparasitic litter additions alter gross nitrogen turnover in temperate semi-natural grassland soils. Soil Biology and Biochemistry 68: 419–428. http://dx.doi.org/10.1016/j.soilbio.2013.10.025

Ducharme L., Ehleringer J. (1996) Gas exchange, $\delta^{13}C$, and the heterotrophy for Castilleja linariifolia and Orthocarpus tolmiei, facultative root hemiparasites on Artemisia tridentata. The Great Basin Naturalist 56: 333–340.

Gasser M., Hammelehle A., Oberson A., Frossard E., Mayer J. (2015) Quantitative evidence of overestimated rhizodeposition using ^{15}N leaf-labelling. Soil Biology and Biochemistry 85: 10–20. http://dx.doi.org/10.1016/j.soilbio.2015.02.002

Gibson C., Watkinson A. (1991) Host selectivity and the mediation of competition by the root hemiparasite Rhinanthus minor. Oecologia 86: 81–87. http://dx.doi.org/10.1007/BF00317393

Govier R.N., Nelson M.D., Pate J.S. (1967) Hemiparasitic nutrition in Angiosperms. I. The transfer of organic compounds from host to Odontites verna (Bell.) Dum. (Scrophulariaceae). New Phytologist 66: 285–297. http://dx.doi.org/10.1111/j.1469-8137.1967.tb06006.x

Gylfadóttir T., Helgadóttir Á., Høgh-Jensen H. (2007) Consequences of including adapted white clover in northern European grassland: transfer and deposition of nitrogen. Plant and Soil 297: 93–104. http://dx.doi.org/10.1007/s11104-007-9323-4

Heide-Jørgensen H.S. (2008) Parasitic flowering plants. Leiden, Brill. http://dx.doi.org/10.1163/ej.9789004167506.i-438

Hertenberger G., Wanek W. (2004) Evaluation of methods to measure differential ^{15}N labeling of soil and root N pools for studies of root exudation. Rapid Communications in Mass Spectrometry 18: 2415–2425. http://dx.doi.org/10.1002/rcm.1615

Hinsvark O., Wittwer A., Tukey H. (1953) The metabolism of foliar-applied urea. I. Relative rates of $^{14}CO_2$ production by certain vegetable plants treated with labeled urea. Plant Physiology 28: 70–76. http://dx.doi.org/10.1104/pp.28.1.70

Jiang F., Jeschke W.D., Hartung W. (2003) Water flows in the parasitic association Rhinanthus minor/Hordeum vulgare. Journal of Experimental Botany 54: 1985–1993. http://dx.doi.org/10.1093/jxb/erg212

Jiang F., Jeschke W.D., Hartung W. (2004) Solute flows from Hordeum vulgare to the hemiparasite Rhinanthus minor and the influence of infection on host and parasite nutrient relations. Functional Plant Biology 31: 633–643. http://dx.doi.org/10.1071/FP03225

Khan D.F., Peoples M.B., Chalk P.M., Herridge D.F. (2002) Quantifying below-ground nitrogen of legumes. 2. A comparison of 15N and non isotopic methods. Plant and Soil 239: 277–289. http://dx.doi.org/10.1023/A:1015066323050

Ledgard S.F., Freney J.R., Simpson J.R. (1985) Assessing nitrogen transfer from legumes to associated grasses. Soil Biology and Biochemistry 17: 575–577. http://dx.doi.org/10.1016/0038-0717(85)90028-8

McNeill A.M., Zhu C., Fillery I.R.P. (1997) Use of in situ ^{15}N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil-plant systems. Australian Journal of Agricultural Research 48: 295–304. http://dx.doi.org/10.1071/A96097

Pageau K., Simier P., Naulet N., Robins R., Fer A. (1998) Carbon dependency of the hemiparasite Striga hermonthica on Sorghum bicolor determined by carbon isotopic and gas exchange analyses. Australian Journal of Plant Physiology 25: 695–700. http://dx.doi.org/10.1071/PP98047

Pageau K., Simier P., Le Bizec B., Robins R.J., Fer A. (2003) Characterization of nitrogen relationships between Sorghum bicolor and the root-hemiparasitic angiosperm Striga hermonthica (Del.) Benth. using $K^{15}NO_3$ as isotopic tracer. Journal of Experimental Botany 54: 789–799. http://dx.doi.org/10.1093/jxb/erg081

Parker C. (2009) Observations on the current status of Orobanche and Striga problems worldwide. Pest Management Science 65: 453–459. http://dx.doi.org/10.1002/ps.1713

Pate J.S., Bell T. (2000) Host associations of the introduced annual root hemiparasite Parentucellia viscosa in agricultural and bushland settings in Western Australia. Annals of Botany 85: 203–213. http://dx.doi.org/10.1006/anbo.1999.1018

Press M.C., Shah N., Tuohy J.M., Stewart G.R. (1987) Carbon isotope ratios demonstrate carbon flux from C_4 host to C_3 parasite. Plant Physiology 85: 1143–1145. http://dx.doi.org/10.1104/pp.85.4.1143

Press M.C. (1989) Autotrophy and heterotrophy in root hemiparasites. Trends in Ecology & Evolution 4: 258–263. http://dx.doi.org/10.1016/0169-5347(89)90195-X

Press M.C. (1998) Dracula or Robin Hood? A functional role for root hemiparasites in nutrient poor ecosystems. Oikos 82: 609–611. http://dx.doi.org/10.2307/3546383

Pirhofer-Walzl K., Rasmussen J., Høgh-Jensen H., Eriksen J., Søegaard K., Rasmussen J. (2012) Nitrogen transfer from forage legumes to nine neighbouring plants in a multispecies grassland. Plant and Soil 350: 71–84. http://dx.doi.org/10.1007/s11104-011-0882-z

Putz B., Drapela T., Wanek W., Schmidt O., Frank T., Zaller J.G. (2011) A simple method for in situ-labelling with ^{15}N and ^{13}C of grassland plant species by foliar brushing. Methods in Ecology and Evolution 2: 326–332. http://dx.doi.org/10.1111/j.2041-210X.2010.00072.x

Pywell R.F., Bullock J.M., Walker K.J., Coulson S.J., Gregory S.J., Stevenson M.J. (2004) Facilitating grassland diversification using the hemiparasitic plant Rhinanthus minor. Journal of Applied Ecology 41: 880–887. http://dx.doi.org/10.1111/j.0021-8901.2004.00940.x

Quested H.M., Callaghan T.V., Cornelissen J.H.C., Press M.C. (2005) The impact of hemiparasitic plant litter on decomposition: direct, seasonal and litter mixing effects. Journal of Ecology 93: 87–98. http://dx.doi.org/10.1111/j.0022-0477.2004.00951.x

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available from http://www.R-project.org/ [accessed 10 Oct. 2014].

Santos-Izquierdo B., Pageau K., Fer A., Simier P., Robins R.J. (2008) Targeted distribution of photo-assimilate in Striga hermonthica (Del.) Benth parasitic on Sorghum bicolor L. Phytochemistry Letters 1: 76–80. http://dx.doi.org/10.1016/j.phytol.2008.01.003

Schmidt O., Scrimgeour C.M. (2001) A simple urea leaf-feeding method for the production of ^{13}C and ^{15}N labelled plant material. Plant and Soil 229: 197–202. http://dx.doi.org/10.1023/A:1004815404197

Seel W., Jeschke W. (1999) Simultaneous collection of xylem sap from Rhinanthus minor and the hosts Hordeum and Trifolium: hydraulic properties, xylem sap composition and effects of attachment. New Phytologist 143: 281–298. http://dx.doi.org/10.1046/j.1469-8137.1999.00461.x

Těšitel J., Plavcová L., Cameron D.D. (2010) Heterotrophic carbon gain by the root hemiparasites, Rhinanthus minor and Euphrasia rostkoviana (Orobanchaceae). Planta 231: 1137–1144. http://dx.doi.org/10.1007/s00425-010-1114-0

Těšitel J., Těšitelová T., Fisher J.P., Lepš J., Cameron D.D. (2015) Integrating ecology and physiology of root-hemiparasitic interaction: interactive effects of abiotic resources shape the interplay between parasitism and autotrophy. New Phytologist 205: 350–360. http://dx.doi.org/10.1111/nph.13006

Manuscript received 12 Feb. 2015; accepted in revised version 24 Aug. 2015.

Communicating Editor: Renate Wesselingh.